RADIOBIOLOGY OF LARGE ANIMALS

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Stanford Research Institute

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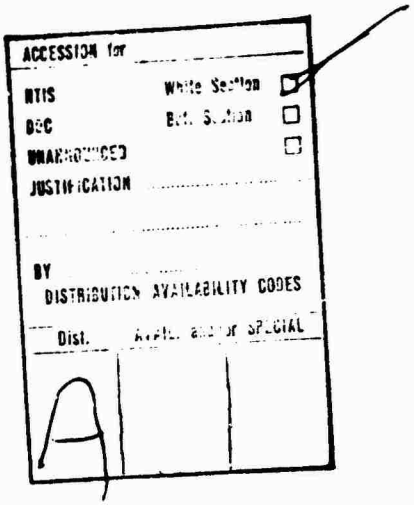

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Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences, National Research Council.

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ABSTRACT

The LD_{50}s for sheep exposed to ^{60}Co gamma rays at 0.9 and 0.45 R/hr (20 and 10 R/day) were 1240 and 1713 R, respectively. Intermittent exposure at an average daily rate (ADR) of 22 to 34 R/day showed that the ADR was the principal parameter determining LD_{50}. Hematological measurements during irradiation showed that the most reliable indicator of radiation injury was the total WBC count. The survival or death of individual sheep could be predicted from their WBC counts with a reliability of about two out of three. Studies of bone marrow showed some aspects of the relationship of bone marrow cell count to radiation lethality and repair of radiation injury. Analysis of data on four mammalian species showed similar relationships of LD_{50} to radiation dose rate (EDR). At high EDRs, the LD_{50} was independent of the EDR. At intermediate values of EDR (30 minutes to 12 hours for lethal exposure), the LD_{50} was a linear function of the EDR. At low values of EDR or ADR (1 day or more for lethal exposure), the average repair rate of the radiation injury was an exponential function of the ADR. The application of the findings for estimation of the lethal effects of radiation on man is discussed.

SUMMARY

The Problem

In the event of a nuclear detonation, human populations will be exposed to ionizing radiations. A significant component of the radiation hazard will be fallout, which produces a wide range of radiation dose rates. The extent, and sometimes the variety, of radiation response depends on both the dose received and the dose rate at which it is delivered. Studies of the interrelationships among dose, dose rate, and response in a variety of animal species, particularly those approximating human size, are the primary source of information on which to base an evaluation of the potential radiation hazard to human populations.

The Findings

Sheep were irradiated with ^{60}Co gamma rays at a variety of dose rates and schedules of intermittent exposure. Dose rates ranged from 660 R/hr to 0.45 R/hr, and schedules of exposure included single, acutely lethal exposures, continuous day-and-night exposures, and intermittent exposures ranging from once daily to once every two weeks. The data collected were mortality of the animals, hematological changes during and after irradiation, and changes in bone marrow cell counts. Experiments in sheep were supplemented with a few experiments in mice for bone marrow and lethality studies. Data on lethality to the sheep relative to dose rate were compared with data on other animal species in the scientific and report literature. A comprehensive analysis of the relationship of lethal dose to rate of radiation exposure was made for the several species studied.

The lethality studies in sheep included direct measurement of the LD_{50}s at 0.9 and 0.45 R/hr (22 hr/day, 20 and 10 R/day). The LD_{50} values were 1250 and 1713 R, respectively. Studies at 3.5 R/hr with

various intermittent schedules of exposure showed that the LD_{50} of the sheep was essentially independent of exposure dose rate at 3.5 R/hr or less, and depended on the average daily rate of exposure instead. Exposures could be averaged over a period of as long as two weeks. Comparable studies at 10.3 R/hr showed that, at this higher exposure dose rate, the LD_{50} probably depends on other factors in addition to the average daily exposure rate. Reirradiation of surviving animals, at 90 or 180 days after completion of the initial radiation exposure studies for LD_{50} determinations, showed that a large component of the radiation injury still had not recovered by 180 days after completion of the original exposure.

Hematological studies during and after exposure to gamma radiation showed that the most consistent and reliable indicator of accumulating radiation injury was the total WBC count. During continuous or intermittent irradiation at 3.5 R/hr or less, the WBC count decreased continuously, reaching an average of about 26% of its mean preirradiation value at the time when the accumulating radiation dose became lethal. Among animals exposed to radiation doses in the midlethal range, survivors had higher average WBC counts than nonsurvivors, independent of the dose received. Based on observation of WBC counts at one week after completion of irradiation, the eventual survival or death of 66 to 73% of the animals could be predicted. For animals surviving long periods after irradiation, the recovery of total WBC count was very slow: The count recovered to about 60 to 80% of its preirradiation value at about one year after irradiation and appeared to stabilize at this level. Total RBC counts were more variable than total WBC counts, both during and after irradiation, and did not appear to reach a stable level until about four weeks after irradiation. Some evidence of hemorrhage was found in the irradiated animals during the postirradiation period, but no evidence was found associating the hemorrhage with death of the animals.

Studies of bone marrow cell count in mice showed that a significant part of the dependence of LD_{50} on radiation dose rate was related to the time after irradiation at which the replacement of the marrow cells began rather than to the extent of survival of the bone marrow cells immediately after irradiation. Studies on bone marrow cell count of sheep during protracted irradiation showed that continued replacement of marrow cells occurred during the course of irradiation and that death of the animals involved subtle effects of the radiation on the dynamics of the marrow replacement rather than on gross destruction of the marrow. The studies in sheep showed also that the marrow cell count was decreased for long times after irradiation; this response corresponded to the long-term depression of total WBC count in blood and the presence of unrecovered lethal injury in the animals.

Analysis of many lethality studies in sheep, pigs, dogs, and mice exposed to a number of schedules of irradiation showed that, when the dose rate was great enough to accumulate a lethal dose in less than 0.5 hour, the LD_{50} did not depend on dose rate for these species. At lower dose rates, or under conditions of intermittent exposure, two principal relationships existed between radiation dose rate and acute LD_{50}. The first relationship occurred when the duration of the radiation to reach a lethal dose was between approximately 0.5 hour and 12 hours. The relationship was of the form

$$LD_{50} = A - B\ EDR$$

where A and B are constants, and EDR is the exposure dose rate (expressed in R/hr) for continuous radiation exposure.

The second relationship between dose rate and LD_{50} occurred when the rate of exposure, including intermittent periods without exposure, was such that the time to reach a lethal dose was about one day or longer. The repair of the radiation injury was related to the rate of accumulation of injury by the relationship

$$R = R_o\ (1 - e^{-k\ ADR})$$

where R is the repair per day, R_o is a constant representing the maximum repair per day, k is a constant, and ADR is the average daily radiation exposure rate (expressed in R/day) over the total exposure period. The LD_{50} can be computed for various dose rates, using the formulae above. For sheep, the values of the constants are as follows: A = 356; B = 0.156; R_o = 24.9; and k = 0.0396. A set of similar, but not identical, constants can be deduced for man. The methods and limitations for obtaining the constants for man are discussed.

ADMINISTRATIVE INFORMATION

The purpose of this Task Order is to continue the research previously conducted under Work Unit No. 2531D of Work Order DAHC20-67-C-0149 at the U.S. Naval Radiological Defense Laboratory. In August 1969, following the Defense Department's decision to close NRDL, the Defense Civil Preparedness Agency (then the Office of Civil Defense) was awarded Stanford Research Institute Contract No. DAHC20-70-C-0219 to continue this work (and three related work units). The Objective and Scope of Work as given in the pertinent Research Task Order attached to that contract are as follows:

> "OBJECTIVE: To improve quantitative models of radiation injury and recovery applicable to man through determination of the effects of protracted gamma irradiation on appropriate mammalian species of large animals in terms of radiation injury, recovery, and physiological alterations."
>
> "SCOPE OF WORK: To evaluate the hazards of nuclear warfare insofar as human population is concerned, it is essential to know more about the biological effects of the protracted irradiation characteristic of exposure in a fallout field. Since in fallout fields the dose rate will range from less than one rad per hour to several hundred rads per hour, our prediction capability must be extended to include the lower dose rate in particular. Information derived from chronic irradiation studies with large domestic animals whose radiation sensitivities and recovery processes are more comparable to those of man would be particularly pertinent."

With the publication and distribution of this final report, all contractual requirements have been satisfied. In every respect, the objectives and scope of the contracted work, as further detailed in the approved Work Plan, have been successfully accomplished and completed on schedule.

CONTENTS

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ILLUSTRATIONS

I INTRODUCTION

This is the final report on this project, which was designed to provide information on which to base estimates of the potential radiation hazards to humans resulting from fallout of nuclear detonations. The major thrust of this program has been toward the development of models for the estimation of hazards from low dose-rate gamma irradiation by studying its effects on large animals, particularly sheep, in terms of accumulation of, and recovery from, injury.

During the current contract year, no new experimental investigations have been undertaken. Instead, the effort has been allocated to: (1) a comprehensive review and analysis of the experimental work already performed on the project, especially the hematological studies in sheep; (2) a review of other studies of the biological effects of ionizing radiation in other large animal species, and a comparison of these studies with the results obtained in the present project, with a view to obtaining maximum generality of information; and (3) the preparation, organization, and writing of this final report.

This report gives an overview of the entire program of experimental studies conducted at SRI, together with the results of data analysis and interpretation. All the actual data from the project at SRI have been presented previously in quarterly and annual reports. Although the data presented here have been condensed and summarized in an effort to make this report of reasonable size, our aim was to make it as comprehensive as possible, given the limitation of space.

The source of ionizing radiation for all experiments consisted of four ^{60}Co sources, each originally 2225 Ci on August 21, 1963. The sources were contained in individual lead shields mounted on carts that could be moved on a set of railroad tracks to various parts of the exposure area. A compressed air-hydraulic system controlled from a remote station raised the sources up out of their shields for exposure of the

animals. For exposure of mice or sheep at high dose rates, the sources were arranged symmetrically around the center of the track system, and the animals were placed in boxes or cages between the sources or at the center of the source system. For exposure of sheep at low dose rates ($\leq$ 10 R/hr), the sources were placed as close together as possible at a convenient point on the track system, and the sheep were confined in individual pens measuring approximately 4 X 4 X 8 ft arranged along an arc at an appropriate distance from the sources. The pens opened onto a runway that provided an easy way of getting sheep into or out of the pens. By selection of the number of sources to be used and of the appropriate distances of the pens from the sources, as many as three experiments could be performed simultaneously. The ^{60}Co exposure range and its equipment were designed and constructed by staff of the U.S. Naval Radiological Defense Laboratory, and the range was located on an army base (Camp Parks) near Pleasanton, California. Descriptions and diagrams of the site have been published.[1]

In setting radiation exposure rates, the locations of radiation sources and animal boxes or pens were approximated, and the dose rates at these locations were measured with Victoreen ionization chambers. The positions of the pens and sources were then readjusted for desired rate and uniformity of exposure, and the final exposure rate was measured again with Victoreen chambers. These chambers had been intercalibrated previously with a chamber calibrated by the National Bureau of Standards. The final determination of each exposure rate involved between 20 and 30 measurements, and the overall standard deviation of the mean exposure rate was between 1 and 2% of the mean. All radiation doses in the present study were expressed as roentgens (R) in air at the midpoint of the animal's position or the midpoint of the pen.

Most of the experiments involved irradiation of sheep, and in most of the sheep experiments, death of the subject was the primary end-point. Hematological measurements were made in most of the sheep experiments, and quantitative measurements of bone-marrow cellularity were made in a few of them. The sheep were Columbia-Rambouillet wethers purchased

from a livestock dealer in Dixon, California. The animals were selected by the dealer for uniform weight (40-50 kg) and appearance of good health. After delivery to the farm at Camp Parks, the sheep were given a routine oral medication series consisting of thiabendazole (two doses at two-week intervals) and triple-sulfa (Thipyrimeth)(one dose on each of three successive days). Blood samples were taken from the sheep after the medication series for preirradiation studies and assignment of the sheep to experimental groups. Sheep were assigned to dose groups in each experiment so that each of the groups initially had similar distributions of total leucocyte (WBC) count. Before irradiation, between sequential exposures, and during the 60-day postirradiation period of observation, the sheep were kept in corrals and fed alfalfa hay. After the 60-day observation period, the sheep were kept in pastures (with hay supplement) until they were required for reirradiation experiments. During irradiation they were fed alfalfa or pellets; water and salt blocks were available at all times.

A few of the experiments involved irradiation of mice. The mice were LAF_1 females purchased from the Jackson Laboratory, Bar Harbor, Maine. The mice were kept in the animal colony at SRI in Menlo Park, and were transported to Camp Parks for irradiation and back to Menlo Park for bone-marrow studies and observations of mortality.

Analysis of the data was performed by conventional mathematical and statistical procedures. The standard reference for analysis of mortality was Probit Analysis, by Finney.[2] The standard reference for other statistical analyses was Introduction to Statistical Analysis by Dixon and Massey.[3]

The major objective of the study was to determine and analyze the relationship between duration or time distribution of radiation exposure and lethal effects of the radiation. Several words or expressions have been used to describe these relationships; these are defined as follows:

(1) The radiation dose rate at the site of the animal during the actual irradiation is called the "exposure dose rate" (EDR).

In the practice of radiology, the EDR is called the "intensity of the radiation," but the term "intensity" is not used in this report. Values of EDR in this report are uniformly expressed as R/hr.

(2) The expressions "acute irradiation" and "chronic irradiation" are defined in terms of the EDR. When the EDR is such that a lethal dose can be reached in one day or less of continuous irradiation, the irradiation is "acute"; otherwise it is "chronic." In the present study, acute irradiation of sheep was at EDRs of 450 to 600 R/hr, and chronic irradiation was at EDRs of 0.4 to 10.3 R/hr.

(3) When the irradiation exposure is distributed over a time period of from one day to several years, it is sometimes referred to as "protracted irradiation." Chronic irradiation is, by definition, protracted irradiation, but protracted irradiation can include intermittent exposure and exposure in the acute range of EDR. The overall dose rate for protracted irradiation in this study is called the "average daily rate" (ADR). Values of ADR are uniformly expressed as R/day.

(4) "Acute lethality," or "acute death," means death occurring within 30 to 60 days after completion of radiation exposure, and resulting from the immediate effects of the radiation on the bone marrow, the gastrointestinal system, and/or the central nervous system. All deaths observed in this study were acute deaths, involving primarily destruction of the bone marrow.

The results of the study are organized in this report as follows: Chapter II presents a summary and analysis of the mortality data; Chapter III is a summary and analysis of the hematological data; Chapter IV contains a summary and analysis of the studies on bone marrow; and Chapter V presents a detailed mathematical analysis of the relationship between mortality of animals and time distribution of the irradiation. Data in Chapters II, III, and IV were derived exclusively from experimental work on the present project. Data in Chapter V include both experimental work in this project and studies performed at other laboratories.

II LETHALITY IN SHEEP EXPOSED TO ^{60}CO GAMMA RADIATION USING VARIOUS EXPOSURE PARAMETERS

The conventional intensity parameter for classifying lethality experiments and describing their results is the exposure dose rate (EDR), i.e., the dose rate at the animal site during the actual irradiation. Although this is certainly appropriate for continuous exposures at high EDRs, it became apparent during this project that another appropriate parameter, particularly for EDRs less than 4 R/hr, is the average dose rate (ADR), i.e., the total dose averaged over the exposure sequence from the beginning of the first irradiation to the end of the last irradiation and including the intervals between increments if the exposure is intermittent. In this chapter, the ADR and the EDR are used, as appropriate, in considering the lethality results for the 21 experiments involving sheep accomplished during this project.

There were five kinds of lethality experiments conducted, varying according to objective. These are described as follows:

(1) A set of six experiments designed to evaluate an earlier finding[4] that a previous exposure at an acute dose rate (A, EDR $\geq$ 30 R/hr) tends to suppress recovery during irradiation at a chronic dose rate (C, EDR < 30 R/hr). There were three experiments (Nos. 1, 3, and 6) of the A-C-A variety involving an initial A exposure immediately followed by a C exposure and then by a final A exposure. The latter was substituted for continuation of the C exposure for operational economy and was used to evaluate the accrued injury at the end of the C exposure by comparison with the results of the other three experiments, each of the A type only (Nos. 2, 3A, and 7).

(2) A set of 12 experiments, 11 of the C variety and 1 of the C-A type, designed to evaluate the relationships among ADR, EDR, and the interval (I) between intermittent exposures. Six of

the experiments (Nos. 5, 8, 11, 12, 19, and 20) involved ADRs from 10 to 30 R/day, EDRs below R/hr, and an I of less than one day. Four experiments (Nos. 13, 15, 16, and 18) involved ADRs from 22 to 24 R/day, an EDR of 3.6 R/hr, and Is of about 5 or 10 days. The last two experiments (Nos. 21 and 23) had ADRs of about 13 R/day. EDRs of about 10 R/hr, and an I of about 13 days.

(3) Two experiments (Nos. 14 and 22) designed to evaluate the effect of dose fractionation by intermittent exposure on lethality at an A dose rate.

(4) One experiment (No. 4) designed to compare survival time during continuous irradiation to death at an EDR of about 4 R/hr with previous comparable data at an EDR of 2 R/hr.[5]

(5) A set of four reirradiation experiments, where 90- or 180-day survivors of previous experiments were exposed again to evaluate the chronology of long-term residual injury and recovery.

Type A Experiments

The results of the three experiments to measure the LD_{50} for exposure of sheep to ^{60}Co gamma rays at high dose rate are shown in Table 1. The experiments were individually conducted in separate batches of sheep over a period of approximately eight months. The LD_{50}s for the first two experiments were close to one another, and the LD_{50} for the third was somewhat higher. The differences in EDR do not appear to be sufficient to account for the higher LD_{50} found in Experiment 7, and, in the absence of any known specific cause, it is assumed to be due to differences among batches of sheep. Confidence limits of 95% for the LD_{50}s (LCL and UCL) are shown.

The mean survival time (MST) at the LD_{50} is derived from the survival times after completion of irradiation of individual decedents. It is obtained by computing the linear regression of individual survival times on total radiation dose and computing the survival time at the LD_{50} from the regression equation. The MSTs are essentially the same

Table 1

MORTALITY, LD_{50}, AND MST IN SHEEP EXPOSED TO SINGLE DOSES OF ^{60}CO RADIATION AT EDRs OF 561-578 R/HR

Dose (R)	Fractional Mortality			
	Experiment 2	Experiment 3A	Experiment 7	Combined
158	0/12	--	--	0/12
193	3/12	1/12	1/12	5/36
229	2/12	2/12	2/12	6/36
265	7/12	9/12	3/12	19/36
301	9/12	8/12	9/12	26/36
337	--	10/12	6/12	16/24
EDR (R/hr)	578	573	561	571
LD_{50} (R)	259	262	302	273
LCL (R)	233	240	263	257
UCL (R)	284	283	340	289
MST (days)	26.1	23.7	25.1	25.4
CI (days)	± 3.3	± 1.6	± 3.7	± 1.6

for all three experiments. Confidence intervals (CI) of 95% for the MSTs are shown. For use in later considerations, the combined LD_{50} and MST data for all three experiments are also tabulated.

Type A-C-A Experiments

The above three A-type experiments serve as the control for the three A-C-A experiments designed to further define the effect of a previous A irradiation on recovery during subsequent C irradiation. In 1969, Still et al.[4] reported that when sheep were exposed to 155 R at an EDR of 510 R/hr, then immediately exposed to variable doses at an EDR of 3.6 R/hr, the total LD_{50} was 326 R. The concurrently measured LD_{50} for an A-only exposure at an EDR of 450 R/hr was 315 R. Comparing these values with those of an earlier study,[1] in which the LD_{50} was 237 R for an A-only exposure at 660 R/hr and 298 R for a C-A exposure of 165 R at 3.9 R/hr and variable doses at 660 R/hr, they concluded that an initial acute exposure reduced the amount of recovery during subsequent chronic exposure.

Data for the three experiments designed to explore this concept further are summarized in Table 2. These three experiments were done in parallel with the above three A experiments (1 with 2, 3 with 3A, 6 with 7) using the same three batches of sheep. The three A-C-A experiments differed with respect to the size of the initial A exposure--45, 9.1, and 0 R for Experiments 1, 3, and 6, respectively. Comparison of the individual pairs of LD_{50}s (Tables 1 and 2) indicates equivalent recoveries of -3, 16, and 11 R for initial A exposures of 0, 9.1, and 45 R, respectively. These results, together with those described above, indicate that previous exposure at a high dose rate may indeed alter recovery during subsequent low dose rate irradiation, but when the dose given at the low dose rate is of the order of 134 to 171 R, the amount of recovery available for modification is so small that definitive modification in terms of relating the extent of alteration to the size of the initial A dose would be difficult to establish. Subjectively, it appears that probably there is still some degree of recovery during

Table 2

MORTALITY, LD_{50}, AND MST IN SHEEP EXPOSED TO ^{60}CO RADIATION ACCORDING TO THE FOLLOWING SCHEDULE: INITIAL DOSE (ID) AT EDRs OF 561-578 R/HR, 134 R AT EDR = 3.8 R/HR, VARIABLE DOSES AT 561-578 R/HR

Total Dose (R)	Fractional Mortality			
	Experiment 6 (ID = 0 R)	Experiment 3 (ID = 9.1 R)	Experiment 1 (ID = 45 R)	Combined
179	0/12	--	--	0/12
219	0/12	2/12	0/12	2/36
259	6/12	2/12	2/12	10/36
300	10/12	9/12	7/12	26/36
340	12/12	10/12	10/12	32/36
381	--	12/12	11/12	23/24
LD_{50} (R)	298	278	270	282
LCL (R)	254	259	281	271
UCL (R)	285	297	316	293
MST (days)	25.1	24.3	22.3	23.6
CI (days)	± 4.3	± 2.8	± 2.5	± 1.8

the C phase of an A-C or A-C-A irradiation, but that the elucidation of the quantitative relationships would require so much experimental effort as to make it unprofitable if not unfeasible. Therefore, it is recommended that for evaluating the consequences of radiation exposure occurring over a relatively short period of time, mixed radiation exposures at high and low dose rates would be best considered by assuming that the entire exposure was given at the high dose rate. This question is discussed further in Chapter V.

ADR Experiments

ADR 10 R/Day, I ≦ 1 Day

Experiment 12 was designed to evaluate lethality at an EDR of about 0.5 R/hr. Since the total exposure time was expected to be too long to be operationally convenient if the experiment were to be done solely in the C mode, the animals were exposed for a total of 74 days at a mean EDR of 0.45 R/hr and were then immediately divided into groups and given a graded series of exposures at an EDR of 514 R/hr.

The results are shown in Table 3. The acute LD_{50} immediately following termination of the exposure of 0.45 R/hr was 157 R compared with an expected 273 R for an A-type irradiation at 514 R/hr with no previous exposure [calculated from Eq. (2) in Chapter V]. For the 74-day C exposure, the total dose was 727 R, and the ADR was 9.83 R/day.

Calculation of the estimated LD_{50} at the latter ADR is based on the assumption that the lethal injury during the C exposure increases as a linear function of the dose. The calculation is shown at the bottom of Table 3. From the acute LD_{50} (157 R), it was estimated that 42.5% of the estimated A-type LD_{50} (273 R) had been accumulated during the 727 R at 9.83 R/day, and hence the estimated LD_{50} for a C-type exposure at 9.83 R/day would be 1713 R with 95% confidence limits of 1281 to 2590 R. The MST at the LD_{50} was 28.5 ± 3.1 days, a value slightly larger than that for A-type exposures (Table 1). The essential validity of the assumption of linear accumulation of lethal injury is analyzed in greater detail in Chapter V.

Table 3

MORTALITY, LD_{50}, AND MST IN SHEEP EXPOSED TO ^{60}CO GAMMA RADIATION FOR 22-23 HOURS DAILY AT AN EDR OF 0.45 R/HR (ADR = 9.83 R/DAY) FOR 74 DAYS (CHRONIC DOSE = 727.4 R) FOLLOWED IMMEDIATELY BY VARIABLE CHALLENGE DOSES AT AN EDR OF 514 R/HR (EXPERIMENT 12)

Challenge Dose (R)	Fractional Mortality
110	3/11
155	7/12
200	7/12
245	8/12
290	10/11
LD_{50} (R)	157
LCL (R)	118
UCL (R)	196
MST (days)	28.5
CI (days)	± 3.1

Estimated LD_{50} at 514 R/hr (R)		273
Fraction at 0.45 R/hr	(272.7 - 156.9)/272.7 = 0.4246	
LCL of fraction	(272.7 - 196.1)/272.7 = 0.2809	
UCL of fraction	(272.9 - 117.8)/272.7 = 0.5680	
Estimated LD_{50} at ADR = 9.83 R/day (R)	727.4/0.4246	= 1713
LCL (R)	727.4/0.5680	= 1281
UCL (R)	727.4/0.2809	= 2590

ADR 20 R/Day, I ≤ 1 Day

Two continuous C-type experiments (Nos. 5 and 11) were conducted at an EDR of about 0.9 R/hr. The results are shown in Table 4. In both of these experiments, the animals were exposed continuously, day and night, except for a 1- to 2-hr period each day when the sources were lowered to allow feeding and watering of the animals and inspection and minor maintenance of the exposure facility. Animal groups were introduced progressively into each experiment, beginning with the largest dose, so that the exposure for all animals was completed on the same day.

In the first experiment, Experiment 5, the doses chosen were too low, and the mortality in the highest dose group did not exceed 17%. Hence, the estimated LD_{50} of 1117 R was considered unreliable. Experiment 11, designed on the basis of the results of Experiment 5, had a satisfactory mortality pattern and showed that the LD_{50} for sheep at this dose rate was 1252 R, with a confidence interval of approximately ± 103 R.

In the period between conducting Experiments 5 and 11, another experiment was performed to explore the effect of radiation exposure at a higher EDR (3.6 R/hr) for a daily time period so that the animals would have an ADR similar to that in Experiment 5. This experiment is shown as Experiment 8 in Table 4. Although, as in Experiment 5, the mortality in the highest dose group did not reach the 50% level, the results show clearly that the LD_{50} for this experiment is essentially the same as that for Experiment 11, and that the lethality of the radiation depends primarily on the ADR, rather than on the EDR.

Inspection of the data in Table 4 indicates that the results of all three experiments can be consolidated. The combined LD_{50} of all three experiments was 1240 R, with a 95% confidence interval of approximately ± 102 R. The combined ADR, weighted for the number of animals in each experiment, was 19.8 R/day, and at this ADR, the LD_{50} is reached after 62.5 days of continuous exposure.

Table 4

MORTALITY, LD_{50}, AND MST IN SHEEP EXPOSED TO ^{60}Co GAMMA RADIATION DAILY FOR 22-23 HOURS (EXPERIMENTS 5 AND 11) OR FOR 5.33 HOURS (EXPERIMENT 8)

	Fractional Mortality			
Dose (R)	Experiment 5	Experiment 11	Experiment 8	Combined
521	--	--	0/12	0/12
669	0/12	--	--	0/12
733	0/12	--	--	0/12
797	1/12	--	--	1/12
810	--	--	1/12	1/12
861	1/12	--	--	1/12
925	2/12	--	--	2/12
942	--	2/12	--	2/12
1,083	--	3/12	--	3/12
1,103	--	--	4/12	4/12
1,223	--	5/12	--	5/12
1,363	--	6/12	--	6/12
1,504	--	11/12	--	11/12
Mean EDR (R/hr)	0.871	0.915	3.63	
Mean ADR (R/day)	19.9	20.1	19.4	
LD_{50} (R)	1,117	1,252	1,251	1,240
LCL (R)	687	1,149	863	1,138
UCL (R)	1,548	1,354	1,639	1,343
MST (days)	--*	19.1	--*	18.7
CI (days)	--*	± 4.8	--*	± 3.6

*Data insufficient for evaluation.

The MST values for Experiments 5 and 8 are essentially meaningless, since they are based on extrapolations from insufficient numbers of animals. The corresponding values for Experiment 11 and for the combined set of experiments indicate that the MST may be slightly lower than that found in the A and A-C-A experiments (Tables 1 and 2).

ADR ~ 30 R/Day, I = 1 Day

Two experiments were performed in which sheep were exposed at a mean EDR of 3.4 R/hr for 41 hr, and then at either 0.9 R/hr (Experiment 19) or 0.45 R/hr (Experiment 20) for 111 hr each week. The total dose was 140 R at 3.4 R/hr and 100 R at 0.9 R/hr (ADR = 34 R/day) or 50 R at 0.45 R/hr (ADR = 27 R/day). The total exposure time was 152 hr each week, i.e., effectively continuous except for about 2-1/4 hr daily for feeding, watering, range maintenance, and shifting animals between the dose rate sites.

The results are shown in Table 5. The total LD_{50}s were 680 R for Experiment 19 and 920 R for Experiment 20. The MSTs were 16.4 and 14.8 days for the two experiments, respectively. Both of these values are significantly lower than those found in acute experiments (Table 1).

For Experiment 19, the LD_{50} was slightly lower than expected. Inspection of the mortality data shows a higher mortality in the 720 R group than might be expected from the general trend in the experiment. If this value had been omitted from the calculation, the resulting LD_{50} value would have been 760 R.

ADR ~ 23 R/Day, I ~ 5 Days

In two experiments, sheep were irradiated at 3.4 R/hr for 41 hr consecutively, and the exposure was repeated at weekly intervals for various fixed total doses. The results are shown in Table 6. For Experiment 13, the LD_{50} was 1016 R, and for Experiment 18 the LD_{50} was 883 R. The discrepancy in the two experiments was rather disappointing, but there is no obvious reason to reject the validity of either one. The two experiments were done one year apart, with Experiment 13 being run concurrently with Experiments 11 and 12.

Table 5

MORTALITY, LD_{50}, AND MST IN SHEEP EXPOSED TO ^{60}CO GAMMA RADIATION AT EDR = 3.4 R/HR for 41 HOURS AND EITHER 0.9 (EXPERIMENT 19) OR 0.45 R/HR (EXPERIMENT 20) FOR 111 HOURS EACH WEEK

	Experiment 19		Experiment 20	
Weeks of Exposure	Total Dose (R)	Fractional Mortality	Total Dose (R)	Fractional Mortality
2	480	3/15	--	--
3	720	11/15	570	1/15
4	960	9/15	760	4/15
5	1,200	13/15	950	6/15
6	--	--	1,140	13/15
Mean ADR (R/day)	34.3		27.1	
LD_{50} (R)	680		920	
LCL (R)	543		823	
UCL (R)	817		1,018	
MST (days)	16.4		14.8	
CI (days)	± 4.9		± 5.4	

Table 6

MORTALITY, LD_{50}, AND MST IN SHEEP EXPOSED TO ^{60}CO GAMMA RADIATION AT EDR = 3.4 R/HR FOR 41 HOURS (140 R) EACH WEEK

	Fractional Mortality		
Dose (R)	Experiment 13	Experiment 18	Combined
560	1/9	--	1/9
840	1/9	4/9	5/18
980	--	5/9	5/9
1,120	6/9	8/8	14/17
1,260	7/9	8/9	15/18
Mean ADR (R/day)	22.9	22.3	22.6
LD_{50} (R)	1,016	883	936
LCL (R)	848	757	836
UCL (R)	1,184	1,009	1,036
MST (days)	17.5	20.0	16.7
CI (days)	± 9.0	± 8.0	± 6.0

The MSTs were 17.5 and 20 days. These values are slightly but not significantly below those for A-type exposure (Table 1).

Experiments 13 and 18 were originally designed to have the same ADRs as Experiments 5, 8, and 11, but it was not realized at the time that the correct interval for calculating the ADR was from the initiation of the first exposure through the end of the last. Thus, although the animals in Experiments 13 and 18 were exposed to 140 R each week, final exposure required only 1.75 days rather than 7, as in Experiments 5, 8, and 11, so that the ADRs in R/day were slightly higher for Experiments 13 and 18, and the LD_{50}s were somewhat lower.

A two-group experiment (No. 15 not tabulated) was conducted at an EDR of 3.4 R/hr for 82 hr consecutively at weekly intervals (ADR = 48 or 54 R/day), but mortality was so high (8/9 and 9/9 at 560 and 840 R, respectively) that the only useful conclusion was that the LD_{50} was less than 560 R.

ADR $\sim$ 24 R/Day, I $\sim$ 10 Days

In Experiment 16, the EDR was 3.4 R/hr, and 280 R exposures were given every two weeks, with even-numbered groups given a final dose of 140 R to make the dose interval between groups 140 R. The pattern of exposure and the mortality results are shown in Table 7. The LD_{50} was 1050 R with a confidence interval of $\pm$ 91 R, the MST was 12.8 days, and the ADR was 23.9 R/day. The LD_{50} was higher than those of Experiments 13 or 18, (ADR $\sim$ 23 R/day, I $\sim$ 5 days, Table 5) but still significantly lower than those for daily irradiation at ADR $\sim$ 20 R/day (Table 4). The MST was significantly lower than that of acutely irradiated sheep.

A curious feature of the results of Experiment 16 is that the mortality of the even-numbered groups, in which the sequence of 280 R exposures was followed by an exposure of 140 R, did not appear to be different from that of the odd-numbered groups, which consisted of the same number of 280 R exposures without the terminal 140 R exposure. The results suggest that the final 140 R exposure had no effect on the mortality of the animals. In Table 7, the LD_{50}s of the odd-numbered and even-numbered groups

Table 7

MORTALITY, LD_{50}, AND MST IN SHEEP EXPOSED TO ^{60}CO GAMMA RADIATION AT EDR = 3.4 R/HR FOR 82 HOURS EVERY TWO WEEKS (280 R/2 WK, ADR = 23.9 R/DAY) WITH TERMINAL DOSES OF 140 R IN EVEN-NUMBERED GROUPS (EXPERIMENT 16)

Group	Exposure Pattern	Total Dose (R)	Fractional Mortality
1	2 x 280 R	560	0/12
2	2 x 280 R + 140 R	700	0/11
3	3 x 280 R	840	3/11
4	3 x 280 R + 140 R	980	3/12
5	4 x 280 R	1,120	8/12
6	4 x 280 R + 140 R	1,260	9/12

	All	Odd	Even
LD_{50} (R)	1,060	997	1,113
LCL (R)	968	866	1,007
UCL (R)	1,151	1,128	1,220
MST (days)	12.8	18.1	14.5
CI (days)	± 2.8	± 3.1	± 5.4
ADR (R/day)	23.9	25.6	22.1

are calculated separately. For the odd-numbered groups, the LD_{50} was 997 R, and the mean ADR was 25.6 R/day; for the even-numbered groups, the LD_{50} was 1113 R, and the mean ADR was 22.1 R/day. Although the difference in LD_{50}s is not formally significiant, the two values are used separately in a subsequent chapter.

ADR ∿ 13 R/Day, I ∿ 13 Days

Two experiments were performed at an EDR of 10.3 R/hr. The exposure was 153 R (15 hr) every two weeks. The results are shown in Table 8 as Experiments 21 and 23. In Experiment 21, deaths began before the anticipated time (between the fifth and sixth increments), and the experimental protocol was altered. Just before the sixth increment, 14 of the 49 survivors were retired from further exposure, and the remaining 35 received the sixth increment. Just before the seventh increment, 13 of the 24 survivors were retired, and the remaining 11 received the seventh increment. Since some of the mortality in animals receiving subsequent increments would be attributable to the earlier increments, the mortality of retired animals was used to estimate the necessary adjustment. Finally, for weighting purposes in computing the LD_{50}, the decimal mortality was converted to fractional mortality with the sum of the actual decedent and retired animals as the denominator. The adjustment formula for mortality following five or six increments is shown in Table 8. Although the adjustment process is acceptable for computing LD_{50}, the MST is not calculable since the individual decedents where deaths would have occurred even without subsequent exposures cannot be identified.

The LD_{50} for Experiment 21 was 813 R, and that for Experiment 23 was 638 R. Both of these values were significantly lower than the LD_{50}s for Experiment 16, where I was similar but the EDR and ADR were lower (Table 7). The combined LD_{50} for Experiments 21 and 23 was 773 R. The results suggest that periodic irradiation experiments at an EDR of 3.5 R/hr give mortality results that are not grossly inconsistent with those at an EDR of 0.9 R/hr, but that periodic irradiation at an EDR of 10 R/hr leads to greater mortality than would be expected on the basis of the ADR. The nature of this difference is analyzed in Chapter V.

Table 8

MORTALITY, LD_{50}, AND MST IN SHEEP EXPOSED TO ^{60}CO GAMMA RAYS AT EDR = 10.2 R/HR FOR 15 HOURS EVERY TWO WEEKS

$$FS = \frac{[B + C]\,[B + (D/C)(A - B)]/A}{B + C}$$

Where FS = Fractional survival
A = Number exposed
B = Number dead before next exposure
C = Number retired
D = Number 60-day retired decedents

Number of Exposures	Total Dose (R)	Experiment 21 Data				FS		
		A	B	C	D	Experiment 21	Experiment 23	Combined
4	612	--	--	--	--	--	5/9	5/9
5	765	67	18	14	2	11.9/32*	5/9	16.9/41
6	918	35	11	13	8	17.7/24*	7/9	24.7/33
7	1,071	11	--	11	7	7/11	9/9	16/20
		Mean ADR (R/day)				13.2	13.5	13.4
		LD_{50} (R)				826	638	745
		LCL (R)				732	482	640
		UCL (R)				920	795	850
		MST (days)				--	27.1	--
		CI (days)				--	± 7.5	--

*Computed from the above formula.

Periodic A-Type Experiments

A preliminary experiment was performed to determine whether there was any recovery in sheep from closely spaced consecutive doses of gamma rays at the high dose rate. The experiment (Experiment 14, not tabulated) consisted of two groups of eight sheep each, exposed to 100 R/day at 512 R/hr for three or five consecutive days. The mortality was 2/8 and 8/8 respectively. The LD_{50} for this study cannot be computed in a conventional way; however, the LD_{50} can be estimated as follows: The mortality of 2/8 for the 300 R exposure gives a probit of 4.326; the mean slope for acute experiments (involving dose rates of > 500 R/hr) was 3.8333 probit units/ln cycle; by extrapolating from 300 R and probit 4.326 to probit 5.000, the expected dose would be 358 R for the LD_{50}. Similarly, the expected mortality at 500 R would be 90%, and the 8/8 mortality found would be reasonable. The expected LD_{50} for a single exposure at 512 R/hr is 276 R, and thus a significant reduction in lethal effect occurs for A-type irradiation fractionated daily over a period of three to five days.

In a second experiment (Experiment 22) to measure the effect of dose fraction ' .n at high dose rates, groups of sheep were exposed to doses of 34, 44, and 54 R at 607 R/hr each Monday, Wednesday, and Friday for five weeks. The results are shown in Table 9. The LD_{50} was 522 R, the MST was 16.8 days, and the expected single dose LD_{50} was 261 R. Hence, repair of radiation injury must have taken place. The repair amounted to approximately 17.4 R/exposure, or 8.16 R/day over the duration of the experiment. It is shown in Chapter V that the average daily repair in Experiment 22 is approximately half the amount expected for C-type irradiation (3.5 R/hr and less). The relationship of the kinetics of repair at high EDR to those at low EDR is discussed in Chapter V.

In Experiment 22, the expected single-dose LD_{50} was 261 R, and the measured LD_{50} was 522 R; hence, the recovery over an exposure period of 32 days was (522 - 261), or 261 R, exactly 50% of the LD_{50}. A comparison of this result with that of a previous study[6] on the effects of daily high dose rate irradiation of mice is interesting. In the latter study, exposure over a 30-day period resulted in a recovery that was 47.2%

Table 9

MORTALITY, LD_{50}, AND MST IN SHEEP EXPOSED TO ^{60}CO GAMMA RADIATION AT EDR = 607 R/HR IN INCREMENTS OF 34, 44, OR 54 R THREE TIMES A WEEK FOR 5 WEEKS (EXPERIMENT 22)

Dose (R/Exposure)	Total Dose (R)	Fractional Mortality
34	510	5/12
44	660	11/12
54	810	11/12

LD_{50} (R)	522
LCL (R)	438
UCL (R)	607
MST (days)	16.8
CI (days)	± 6.6

Expected single dose LD_{50} for 607 R/hr: 261 R

of the measured LD_{50}, and exposure over a 40-day period resulted in a recovery that was 53.2% of the measured LD_{50}. Hence, the relative recovery in sheep was the same as that found previously for mice.

Reirradiation Experiments

A series of reirradiation experiments were conducted to evaluate the amount of residual injury still present at 90 or 180 days after termination of the original exposures. It should be noted that this effort was regarded as an operationally efficient adjunct and was not allowed to interfere with the primary objectives. Therefore, it was necessary to group survivors for reassignment to reirradiation studies with a consequent lack of precision in experimental uniformity.

Animals surviving chronic irradiation in Experiments 5, 8, and 16 were reassorted into groups at 90 days after completion of the initial radiation exposure, and were given a series of acute doses at EDRs of 465-540 R/hr to evaluate the amount of residual injury still present from the original exposure. Survivors of Experiments 11, 12, and 13 were separated into two groups at 90 days postexposure. The first set was selected from survivors of the higher dose groups in the original experiments, and was further subdivided and reirradiated at 90 days. The second set was similarly subdivided and reirradiated at 180 days. The results for all five reirradiated groups are summarized in Table 10. The residual injury at the time of reirradiation is expressed as a percentage of the injury accrued at the end of the initial exposure. It is computed by subtracting the challenge LD_{50} from the expected (i.e., without previous exposure) LD_{50} for the challenge EDR (see Chapter V), expressing the difference as a percentage of the challenge LD_{50}, and dividing it by the mean initial dose expressed as a percentage of the initial LD_{50}. Thus, if the challenge LD_{50} were 195 R and the expected LD_{50} were 260 R, the remaining injury would be 65 R, or 25% of an LD_{50}. If the mean initial dose were 900 R and the LD_{50} for the study were 1200 R, the mean initial dose would be equivalent to 75% of an LD_{50}. Then, the 25% of an LD_{50} found on challenge would be 25/75, or 33%, remaining from the initial injury at the time of challenge.

Table 10

MORTALITY, LD_{50}, MST, AND RESIDUAL INJURY SHEEP REEXPOSED TO ^{60}Co GAMMA RADIATION AT ACUTE EDRs 90 TO 180 DAYS FOLLOWING INITIAL EXPOSURES AT EDRs OF 0.45-3.4 R/HR

	Fractional Mortality				
	90 Days P.I.				180 Days P.I.
	Experiment Number				
Challenge Dose (R)	5R	8R	11-13R	16R	11-13R
90	--	--	--	3/12	--
130	--	--	5/11	4/12	3/11
140	--	2/11	--	--	--
170	--	--	4/11	7/11	5/12
180	--	6/10	--	--	--
210	9/14	--	11/11	10/12	9/12
220	--	7/10	--	--	--
270	15/15	--	--	--	--
330	15/15	--	--	--	--
Challenge EDR (R/hr)	565	533	497	465	482
Challenge LD_{50} (R)	(188)*	179	(146)*	140	171
Challenge LCL (R)	--	153	--	113	142
Challenge UCL (R)	--	205	--	168	199
Challenge MST (days)	27.4	30.7	28.1	34.9	37.2
Challenge CI (days)	2.2	3.7	3.1	3.8	3.6
Expected LD_{50} (R)	268	273	278	283	281
Residual injury (% LD_{50})	(30)	35	(47)	50	39
Initial injury (% LD_{50})	68	62	106	77	79
Residual injury (% Initial)*	(44)	56	(44)	65	49

*See text.

In Experiment 5R, the challenge doses chosen were too high, and two of the three doses gave 100% mortality. In this case, the challenge LD_{50} was estimated from the probit of mortality at the lowest dose (5.363) and the slope of the dose-response regression line for Experiment 8R (3.217 probit units/ln cycle). Parentheses are used in Table 10 to indicate values not based on the usual regression equation for LD_{50}. In the second study, Experiment 8, the LD_{50} was 179 R, a value not signigicantly different from that estimated for Experiment 5R. The challenge LD_{50} was not calculable from the mortality data for the survivors of Experiments 11-13 reirradiated at 90 days, so a crude estimate was made from an eye-fit plot of the three data points. The crude challenge LD_{50} of 146 R appears somewhat lower than those for Experiments 5R and 8R but is consistent with the computed challenge LD_{50} for Experiment 16R (140 R). Examination of the values for residual injury at 90 days after the initial exposures indicates that it ranges from a low of about 44 to a high of 65% of the accrued injury at the end of the initial exposure, and that it does not appear to depend on the extent of the initial accrued injury. Further, the challenge LD_{50} and the residual injury value of 49% for the animals reirradiated at 180 days after the initial exposure (last column, Table 10) are within the range of the corresponding values for 90-day reirradiation, indicating no discernible change in the extent of residual injury from 90 to 180 days after the initial exposure. Thus, these data indicate that there is some recovery from the initial injury before 90 days after the initial exposure, but little or no further recovery occurred during the subsequent 90 days. Further, taken together, the results imply that following termination of chronic irradiation at the low dose rate, a large part of the lethal injury either does not recover or else recovers only at a very low rate. In a previous study in mice,[7] it was estimated that this nonrecoverable portion of injury amounted to about 10% of the original dose given. Although the present experiments were not originally designed to study repair or lack of repair of radiation injury, the results of the reirradiation experiments indicate that the 10% figure for mice is probably too conservative for sheep, and that perhaps as much as 50% of lethal radiation injury in sheep is nonrecoverable.

The MSTs after reirradiation ranged from 27.4 to 37.2 days. These survival times are consistently and significantly longer than those found after acute irradiation (Table 1). The result suggests that, in this case, in addition to having a lower LD_{50}, the reirradiated animals remain vulnerable to dying for a longer period of time after irradiation. This interpretation would be consistent with studies reported previously by Baum and Alpen[8] and Hendry and Lajtha,[9] showing that repeated doses of X-rays in rats and mice caused progressively increasing delays in the repopulation of the bone marrow.

In a final experiment designed to evaluate residual injury as estimated by chronic reirradiation, surviving animals from Experiment 20 were exposed at an EDR of 10.5 R/hr for 11.5 hr once each week for four, five, six, or seven weeks, beginning at 90 days after termination of the original exposure. The results are shown in Table 11. The challenge LD_{50} was 533 R, the ADR was 21.3 R/day, and the mean dose received in the previous exposure was 82% of the LD_{50} (755 R). The MST was 19.8 ± 6.2 days. This value is slightly less than that found for acute radiation and is consistent with the MSTs of other chronically irradiated animals. Unfortunately, there was no initial experiment where all three exposure parameters (ADR, EDR, and I) were similar enough to those used in Experiment 20R to provide a precise estimate of the expected LD_{50}, which is the basis for estimating residual injury. Since in the discussion of Experiments 21 and 23 (Table 8), it was noted that lethality following an EDR of about 10 R/hr was not consistent with lethality after EDRs of less than 4 R/hr, the expected LD_{50} for Experiment 20R was estimated by adjusting the combined LD_{50} value for Experiments 21 and 23 (see Chapter V).

The residual injury value of 20% estimated by chronic reirradiation appears markedly lower than those derived from acute reirradiation (Table 10). Although this single estimation of residual injury at 90 days by chronic reirradiation and the single estimate of residual injury at 180 days by acute irradiation (Table 10) are insufficient for definitive conclusions, some speculations are of interest. From a comparison of the acute reirradiation results (Table 10), it appears that the on-going

Table 11

MORTALITY, LD_{50}, MST, AND RESIDUAL INJURY IN SHEEP REEXPOSED TO ^{60}CO GAMMA RADIATION AT EDR = 10.5 R/HR for 11.5 HOURS EACH WEEK 90 DAYS FOLLOWING INITIAL EXPOSURES AT EDRs OF 0.45 - 3.4 R/HR (EXPERIMENT 20R)

Number of Challenge Exposures	Total Challenge Dose (R)	Fractional Mortality
4	480	3/9
5	600	4/9
6	720	9/9
7	840	9/9

Challenge EDR (R/hr)	10.5
Challenge LD_{50} (R)	553
Challenge LCL (R)	491
Challenge UCL (R)	616
Challenge MST (days)	19.8
Challenge CI (days)	6.2
Expected LD_{50} (R)	659
Residual injury (% LD_{50})	16%
Initial injury (% LD_{50})	82%
Residual injury (% Initial)	20%

rate of recovery per unit time is very low during the 90- to 180-day period following the initial exposure. If it is assumed that there would have been a similar value for residual injury in Experiment 20R if the reirradiation had been of the acute type, then the finding of a residual injury of only 20% appears paradoxical in the sense that residual injury is not a constant determined by the characteristics of the previous radiation history and the elapsed time, but it is somehow a function of the particular technique used to measure it. In the present context, the explanation may lie in the phenomenon of recovery during chronic exposure, which is thought to account for the higher LD_{50}s associated with chronic exposure compared with acute irradiation. Chronic irradiation, in general, appears to activate recovery processes, while absence of irradiation leads to stagnation and arrest of recovery processes; these points are well illustrated by the chronic irradiation and reirradiation experiments described previously. In the case of reirradiation with a chronic irradiation protocol, the chronic reirradiation appears to reactivate recovery processes in the animal so as to offset in part (although not completely) the injury remaining unrecovered from the previous chronic irradiation.

C-Type Irradiation to Death

A group of 24 sheep was exposed to ^{60}Co gamma rays at an EDR of 3.8 R/hr to evaluate the role of EDR in the effect of continuous irradiation on the survival time following completion of a lethal exposure. The results are shown in Table 12. There was a narrow distribution of survival times with the earliest death occurring at 22 days, the last at 28 days, and an average survival time (AST) at 24.7 days. Previous work by Page et al.[10] showed that the LD_{50} for sheep exposed to predetermined doses at an EDR of 3.6 R/hr was 495 R with an MST of 22.4 ± 3.0 days. If it is assumed that the LD_{50} for the present exposure was accrued at the same point as that determined by Page et al., it would have been completed at 5.66 days, and the AST using the LD_{50} time as the base would have been 28.1 days. Thus, irradiation beyond the estimated LD_{50} reduced the AST by 3.4 days or about 15%. It should be noted that shortening

Table 12

MORTALITY IN SHEEP EXPOSED CONTINUOUSLY (23 HR/DAY) TO ^{60}CO GAMMA RADIATION AT 3.8 R/HR (EXPERIMENT 4)

Number of Animals	Survival (Days)	Accrued Dose (R)
4	22	1,923
4	23	2,010
4	24	2,098
4	25	2,185
2	26	2,272
4	27	2,360
2	28	2,447

Average Survival Time: 24.5 days

of survival time by "wasted" radiation is consistent with the concept of survival time being an inverse function of dose, since in the irradiation-to-death experiment there are no "low lethal" doses. On the other hand, the results of Still et al.[5] indicate that at an EDR of 1.96 R/hr, the "wasted" radiation does not shorten survival time, since they found survival times of 25 to 60 days with an AST of 43 days. Taken together, the results suggest that the survival time under chronic irradiation to death may be a complex function of the ADR, but the experimental data are not sufficient to identify the nature of the functional relationship.

Summary and Conclusions

This chapter has presented a comprehensive summary of all of the data acquired in this study on the lethal effects of gamma radiation in large animals. The results show generally that when the radiation exposure is distributed over a significant time range, the lethal effectiveness of the radiation is substantially reduced. For exposure at 20 R/day, the median lethal dose in sheep was 1240 R, and at 10 R/day, it was 1713 R. For dose rates of 3.6 R/hr or less, the exposures for average daily rate could be averaged over a period of as long as two weeks.

It is generally assumed that the increase in lethal dose (or reduction in lethal effectiveness) occurs because natural repair processes in the animal during the protracted time of exposure act to offset, in part, the accumulating lethal effect of the radiation. There have been many studies of the kinetics of recovery from lethal radiation effects in a number of different animal species. A significant and interesting finding in the present study is that the recovery process during protracted irradiation depends on the condition of being irradiated. When irradiation has been completed, the recovery process appears to slow down and stop at a condition of incomplete recovery.

The relationship between recovery rate and radiation exposure rate is examined analytically in Chapter V, both for the present study and for results in the literature on other animal species.

III HEMATOLOGICAL FINDINGS IN SHEEP EXPOSED TO LETHAL LEVELS OF ^{60}CO GAMMA RAYS AT VARIOUS DOSE RATES AND UNDER VARIOUS CONDITIONS OF EXPOSURE

Systematic hematological studies were made before, during, and after irradiation in all of the sheep used in the lethality studies described in Chapter II, except Experiment 14. Blood samples were taken from the jugular vein in heparin-moistened syringes. Erythrocyte (RBC) and leucocyte (total WBC) counts were made with electronic (Coulter) counters. Packed cell volumes (PCV: hematocrit) were determined by a micro-hematocrit method, using capillary tubes and a special centrifuge. Blood smears stained with Wright's stain were counted under high magnification (oil) to determine percentages of granulocytic and mononuclear cells. The percentages were based on counting 100 cells per slide, and further differential counting was not done. Data on each animal for each sample were tabulated by experiment, group, and day of sample, and percentages of granulocytic and monocytic cells were converted to respective numbers of cells per cubic millimeter of blood by multiplying the percentage of cells by the total WBC count.

Before beginning each experiment, preirradiation blood samples were taken and counted, and animals were assigned to groups of the experiment in such a way as to produce the same mean and variance of total WBC count in each group. During chronic irradiation, blood samples for hematology were taken at weekly intervals in the group that was scheduled to receive the highest total dose of radiation. Following all radiation experiments (except Experiment 14), blood samples were taken in all experimental groups at weekly intervals for 8 weeks. In some experiments, samples were also taken at 12 weeks, and in two selected groups of two experiments, sampling was continued at 4-week intervals for 62 weeks.

Data presented in this chapter have been presented before, usually in a different form, in previous quarterly and annual reports for this project. The format used here was chosen to show the broadest generalization of results across a number of different experiments. In addition, the material in this chapter is organized to give information on the relationship between radiation dose or lethality and peripheral blood counts in such a way that some diagnostic and prognostic judgments can be made from blood counts under field conditions. Finally, the data presented do not always represent all groups and experiments of a given class. Where data have been selected for presentation from a larger pool, the selection has always been made to include a sufficient number of the most representative experiments to show the generality of the findings.

General Characteristics of Hematological Findings in Sheep

For the sheep used in these studies, the average preirradiation values of the hematological parameters were: RBC, 11.3 million cells/mm^3; PCV, 38%; WBC, 7400 cells/mm^3; granulocytes, 2100 cells/mm^3; and monocytes, 5300 cells/mm^3. A previously published report[11] on the hematological findings in unirradiated sheep showed that (except for PCV) the hematological parameters were not normally distributed, that the distribution was frequently skewed toward the high side, and that significant differences occurred among all hematological parameters for different batches of sheep.

In the course of analysis of radiation effects on the WBC count, it was noted that, as the mean WBC counts in chronic irradiation experiments decreased during irradiation, the standard deviations of the means also appeared to decrease in proportion to the means. The relationship between standard deviation of the mean count and the mean itself is shown for Experiments 11, 13, 18, 19, and 20 in Figure 1. The points show considerable scattering, but the trend is clear. The dashed line in the figure was computed by linear regression of the standard deviation on the mean, and it appears to represent adequately the trend of the data. However, since the distribution of standard deviations is chi-square rather than normal, confidence intervals for the lines cannot be drawn.

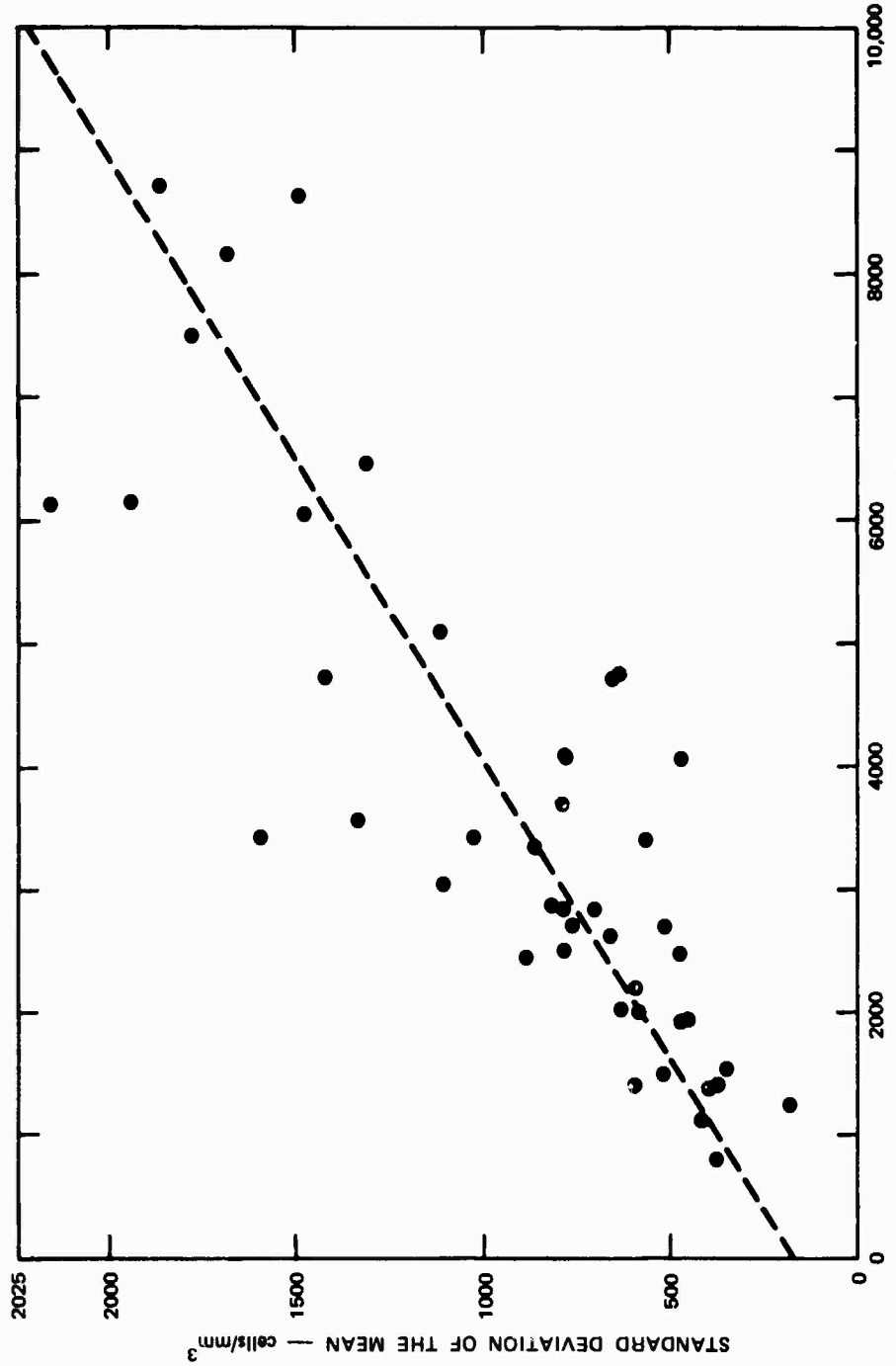

FIGURE 1 RELATIONSHIP OF MEAN WBC COUNT TO STANDARD DEVIATION OF THE MEAN IN SHEEP

Figure 2 shows the corresponding relationships of standard deviation to mean count for granulocytic cells (open circles) and monocytic cells (solid dots) for the same experiments represented in Figure 1. The solid line, representing the trend in granulocytic cells, and the dashed line, representing the trend in monocytic cells, were also computed by linear regression of the standard deviation on the mean count. It is evident that the standard deviation is a considerably higher proportion of the mean count for granulocytes than for monocytes, and the granulocyte counts are hence inherently more variable than monocyte counts.

The slope of the line in Figure 1 is 0.202 for the total WBC count. The slope of the dashed line in Figure 2 is 0.262 for the monocyte count, and the slope of the solid line is 0.538 for the granulocyte count. These slopes represent the average ratio of the standard deviation to the mean count for each of these hematological parameters over a wide range of radiation doses. For unirradiated controls only, the average ratios of standard deviation to mean count were: total WBC, 0.266; monocytic cells, 0.305; and granulocytic cells, 0.612.

In some groups of the chronic irradiation experiments, two measurements were made of the hematological parameters before beginning irradiation. The time intervals between measurements ranged from one to four weeks. Using these paired values, an analysis was made of the sources of variation in hematological measurements in the following manner: The pooled value of the variance within groups was computed for all experimental groups for each hematological parameter. Then the pooled value of the variance for each pair of measurements for each animal was computed across all experimental groups. The first calculation represents the overall variance of the hematological parameters from all sources for a typical group of sheep, and the second calculation represents the variance arising from errors of measurement and from random and uncontrolled causes.

The results of the analysis are shown in Table 13. For total WBC, the overall variance within groups was about 2.9 million, and the pooled variance for remeasurement within animals was slightly over 1 million.

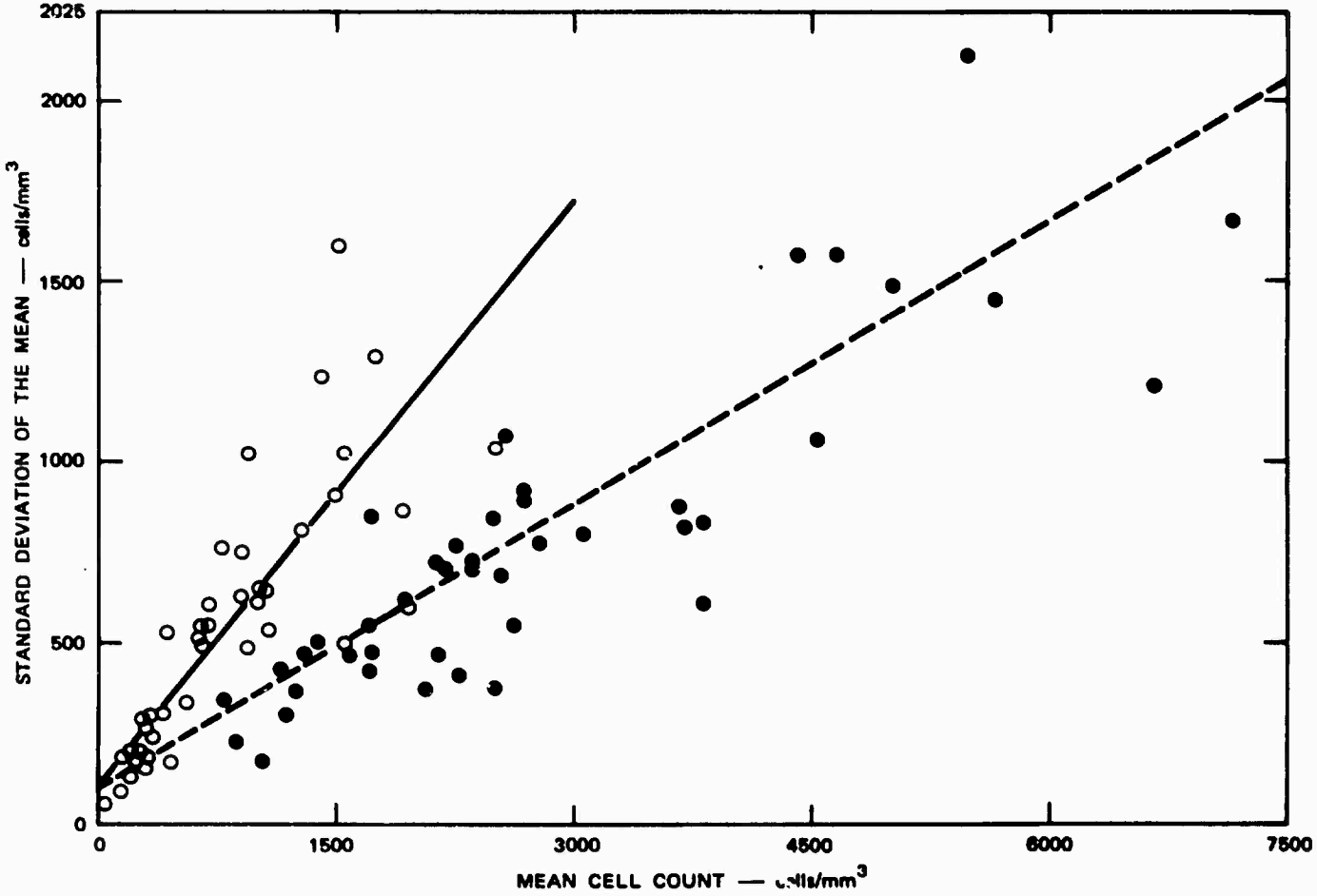

FIGURE 2 RELATIONSHIP OF MEAN COUNTS OF GRANULOCYTIC CELLS (OPEN CIRCLES) AND MONOCYTIC CELLS (CLOSED CIRCLES) TO STANDARD DEVIATIONS OF THE MEAN IN SHEEP

Table 13

ANALYSIS OF THE SOURCES OF VARIATION IN HEMATOLOGICAL PARAMETERS IN SHEEP
(EXPERIMENTS 8, 18, 19, AND 20)

	Total WBC (cells/mm^3)	Granulocytes (cells/mm^3)	Monocytes (cells/mm^3)	RBC (cells/mm^3 x 10^{-4})
Overall mean count	6,331	1,307	5,023	1,126
Overall pooled variance within groups (x 10^{-5})	29.2	7.02	20.3	0.21
Overall standard deviation	1,710	838	1,423	145
Standard deviation/mean	0.270	0.641	0.283	0.129
Pooled variance for test/retest within animals (x 10^{-5})	10.8	6.19	9.97	0.16
Standard deviation for test/retest	1,039	787	999	125
Standard deviation/mean	0.164	0.602	0.199	0.111
Estimated variance for measurement (x 10^{-5})	0.27	1.10	3.45	0.06
Residual variance (x 10^{-5})	18.5	0.82	10.3	0.05
Residual standard deviation	1,358	287	1,014	74
Standard deviation/mean	0.216	0.219	0.202	0.0658

The corresponding standard deviations [standard deviation = (variance)$^{1/2}$] were 1710 and 1038 cells/mm^3, respectively. To express the results simply, if we measured the total WBC in 12 sheep, we would expect a typical variance of 2.9 million, and a standard deviation of around 1700 cells/mm^3 for the group as a whole; if we measured the total WBC in one sheep 12 times at weekly intervals, we would expect a typical variance of around 1 million, and a standard deviation of around 1000 for the mean of the set of measurements.

The calculations in Table 13 show that, although the test/retest variance is about one-third of the total variance for the total WBC count, it is about one-half of the total variance for the monocyte count and 85% of the total variance for the granulocyte count. The test/retest variance can be considered to comprise a variance for the accuracy of measurement and a variance for the instability of the hematological parameter. The measurement variance for the total WBC count was estimated as follows:

Contributing Factor	Value	Variance Contribution
Total cell count	6,330	6,330
Mean range of accepted counts	70	4,900
Pipetting errors	2%	16,030
Total		27,260

The measurement variances for the granulocyte and monocyte counts were calculated as the value of the WBC count variance plus a variance calculated from the fact that the calculated count in each animal is based on the actual observation of an average of only 20 granulocytic and 80 monocytic cells. The estimated measurement variances for granulocytes and monocytes are large, but are still only one-sixth and one-third of the total test/retest variances. It can be concluded that, certainly in large-scale hematological testing, the major source of variability in hematological studies on an individual subject is the inherent instability of the hematologic parameters, and not the procedures of measurement.

The residual variance, as shown in Table 13, is the difference between the overall pooled variance within groups and the pooled test/retest variance. This variance is the variance among animals. The computed ratios of standard deviation to mean for these variances are rather similar, which implies that the differences in variability among these hematological parameters reflect problems of measurement and instability, rather than gross differences among animals.

Calculations are also shown in Table 13 for variance of RBC count. As the numbers show, the variability of the RBC count is considerably less than that of the WBC count. Perhaps the most surprising result is the evidence that the largest variance was that for instability of the RBC count within the animal.

Leucocyte Counts During Chronic Irradiation

The problem was to compare a number of chronic radiation experiments in which the pattern of exposure, the daily rate of exposure, or the LD_{50} were different. To make the comparison, the accumulated dose in each experiment at the end of each week of exposure was divided by the computed LD_{50} for the experiment, and the resulting fraction of the LD_{50} was used in place of either duration of exposure or accumulated dose (in R). In a similar manner, the mean WBC count in each experiment at each week of measurement was divided by the mean preirradiation value of WBC count for all of the chronic irradiation experiments. The fraction of preirradiation WBC count was plotted against the fraction of LD_{50}, and the results are shown in Figure 3. The plot shows a definite and continuous relationship between accumulated lethal effect of the radiation and decrease of WBC count, which reaches about 60% of the preirradiation value when 20% of the LD_{50} has been given. The slope of the trend appears to decrease continuously, and when the LD_{50} has been reached, the WBC count stands at about 25% of the preirradiation value.

Similar analyses of the monocytic and granulocytic cell counts are shown in Figures 4 and 5, respectively. The trend of monocytic cell counts during chronic irradiation is essentially similar to that of

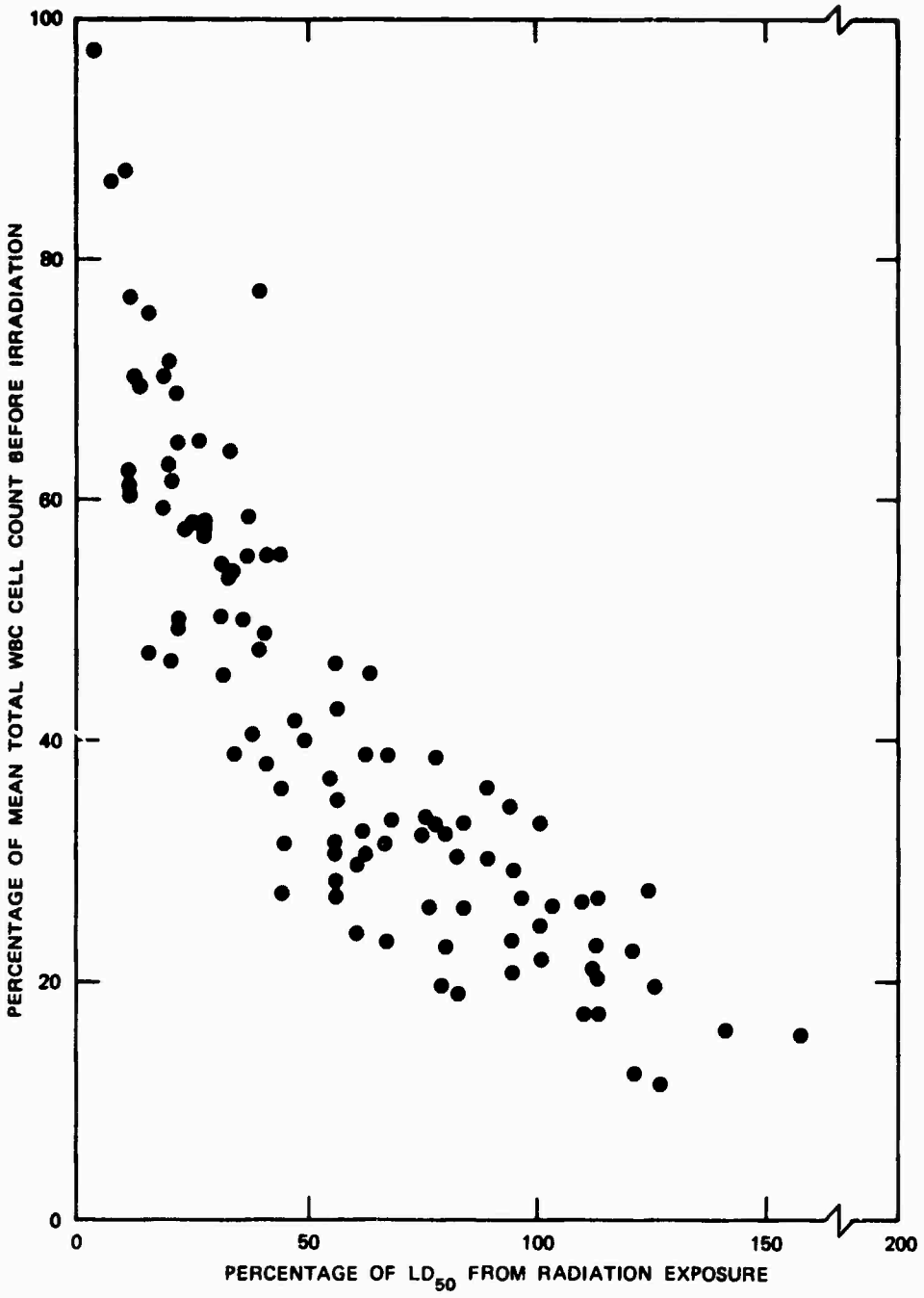

FIGURE 3 RELATIONSHIP OF TOTAL WBC COUNT TO ACCUMULATED RADIATION DOSE DURING CHRONIC EXPOSURE TO IONIZING RADIATION

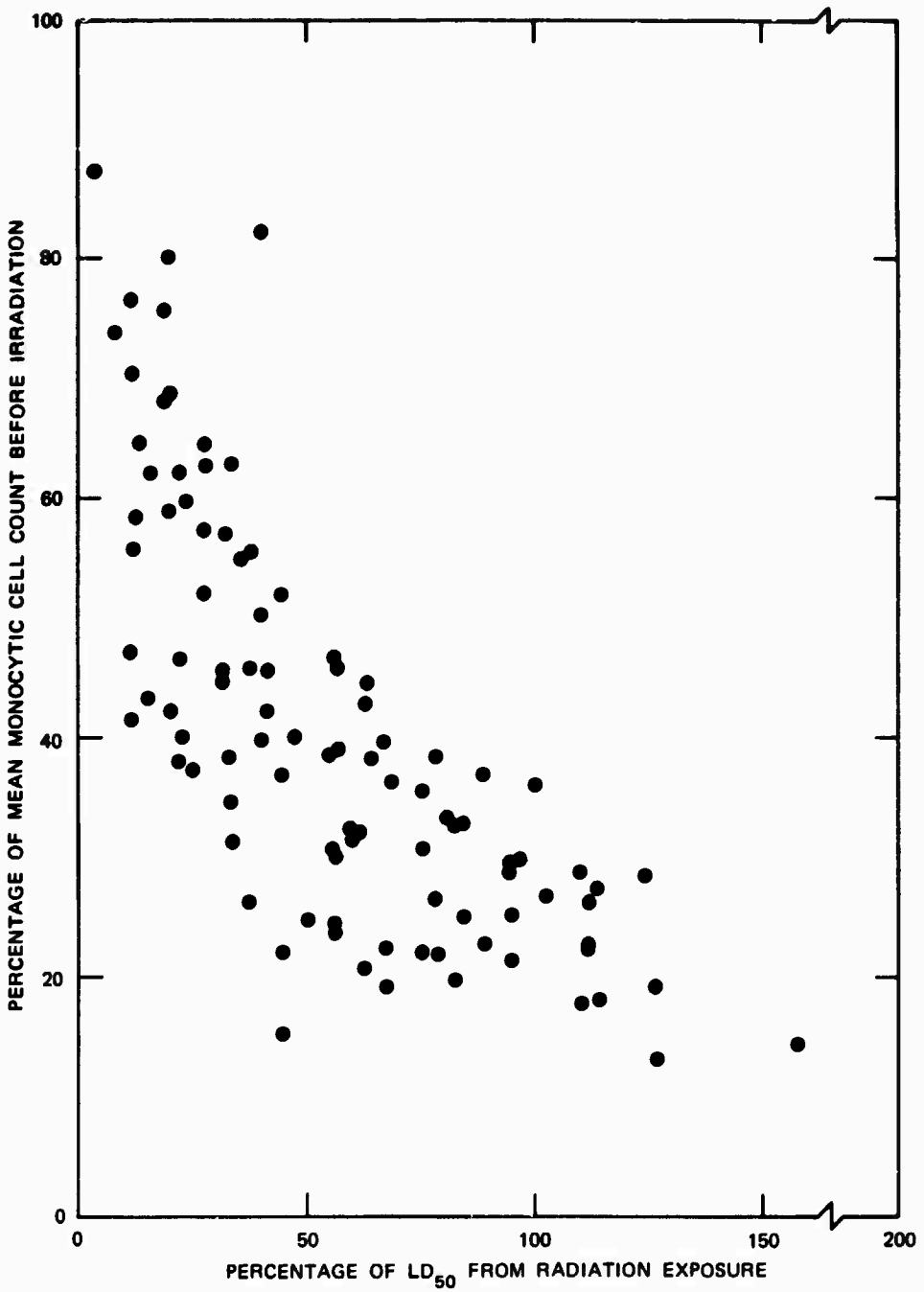

FIGURE 4 RELATIONSHIP OF MONOCYTIC CELL COUNT TO ACCUMULATED RADIATION DOSE DURING CHRONIC EXPOSURE TO IONIZING RADIATION

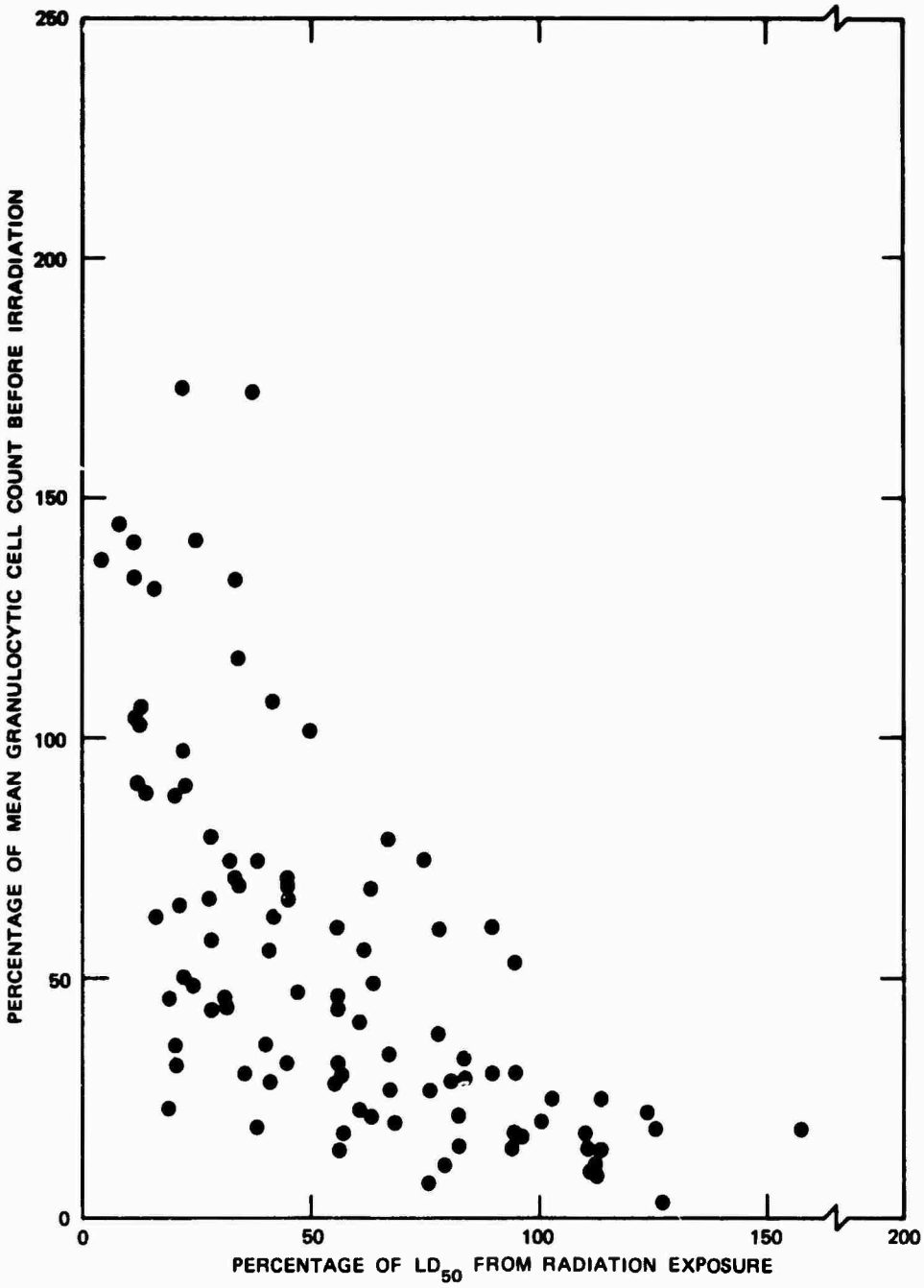

FIGURE 5 RELATIONSHIP OF GRANULOCYTIC CELL COUNT TO ACCUMULATED RADIATION DOSE DURING CHRONIC EXPOSURE TO IONIZING RADIATION

total WBC counts, but the scattering of the points is greater because of the greater variability of monocytic cell counts. The granulocytic cell counts show a peculiar response during chronic irradiation. Up to a total dose of 50% of the LD_{50}, some of the mean cell counts are greater than the mean initial value, as high as 175% of the initial value, while at the same time a few points are depressed to the average level occurring at the LD_{50} (30%). The number of mean cell counts greater than the mean preirradiation value during this period of irradiation was 15 out of a total of 49. Other than the fact that the maximum value of granulocytic cell count decreases with increasing radiation dose (after 40% of the LD_{50} has been accumulated), there appears to be little relationship between total dose and cell count.

It is concluded that the best hematological indicator of increasing lethal injury during exposure to chronic radiation at low dose rate is the total WBC count. The monocytic cell count is more variable than total WBC count, and the granulocytic cell count gives virtually no information about accumulated dose or lethal injury.

The trend of WBC count shown in Figure 3 suggests the possibility of an exponential decrease of total WBC count with increasing radiation dose. Figure 6 shows the WBC count during radiation plotted on a logarithmic scale, against accumulated radiation dose. It can be seen that the trend of cell count was somewhat linear with dose, but the line extrapolated to 75% of preirradiation count at zero dose, leaving out an early, rapid component of cell count loss. Moreover, the scattering of the points appeared to be greater than in the case of the linear plot. We feel that plotting the WBC count on a logarithmic scale against dose offers no real advantage over plotting on a linear scale, and may lead to conceptual oversimplifications in the interpretation of WBC cell count depletion during chronic irradiation.

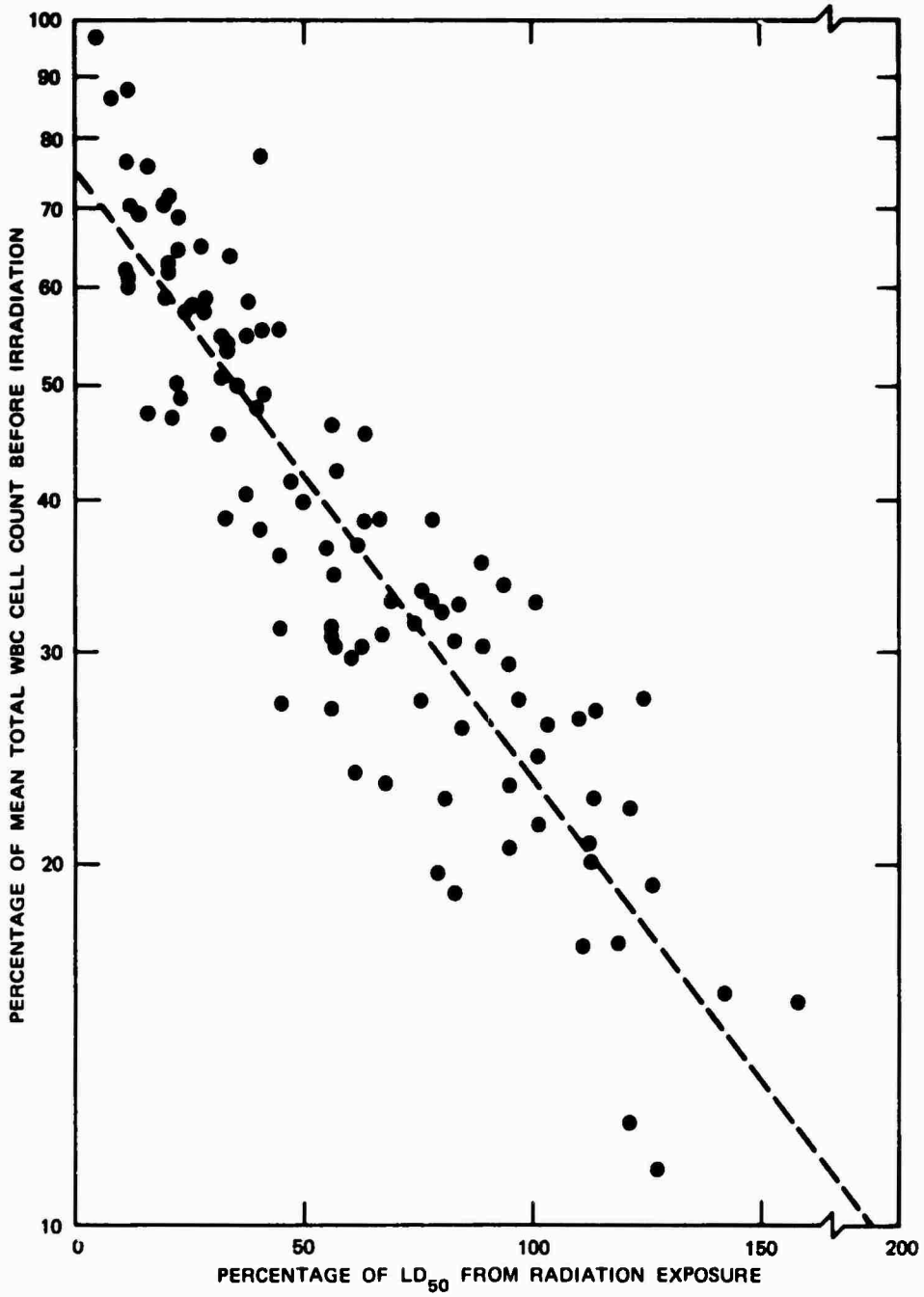

FIGURE 6 RELATIONSHIP OF LOGARITHM OF TOTAL WBC COUNT TO ACCUMULATED RADIATION DOSE DURING CHRONIC EXPOSURE TO IONIZING RADIATION

Leucocyte Counts Following Exposure to Ionizing Radiation

Following termination of chronic irradiation, or following acute or A-C-A irradiation or acute reirradiation, the total WBC count was depressed in the irradiated animals and remained depressed throughout the 60-day postirradiation period of observation. The relationship of total WBC count to radiation dose for the first week after termination of chronic irradiation is shown in Figure 7. There is a weak relationship of WBC count to radiation dose. The dashed line in the figure represents the linear regression of total WBC count on radiation dose. Similar relationships are found for counts of monocytic and granulocytic cells and for total WBC, monocytes, and granulocytes in animals one week after exposure to acute or A-C-A irradiation or acute reirradiation. All the plots share two things in common: a weak dependence of cell count on radiation dose, with wide scattering of points, and an extrapolated value of cell count at zero dose which is significantly, and usually grossly, lower than the mean preirradiation value.

The mean WBC count at the LD_{50} one week after chronic irradiation was 2060 cells/mm^3, about 28% of the mean preirradiation count. The mean WBC counts at the LD_{50} at one week after acute irradiation, A-C-A irradiation, and acute reirradiation were 2430, 2760, and 2170 cells/mm^3, respectively. The corresponding percentages of the mean preirradiation count were about 33, 38, and 29%. Thus, one week after an LD_{50} dose of radiation, the total WBC count averaged between 28 and 38% of its preirradiation value, irrespective of the dose rate or radiation schedule.

For analysis of the pattern of WBC, granulocyte, and monocyte counts in sheep over the eight-week observation period following irradiation, the animals in each of the major categories (acute, A-C-A, chronic, and acute reirradiation) were further subdivided into survivors and nonsurvivors. The means and standard deviations of the cell counts for survivors and nonsurvivors were computed separately for all the experiments in each major category, except that for chronic irradiation the analyses were based on Experiments 11, 13, 16, 18, 19, and 20.

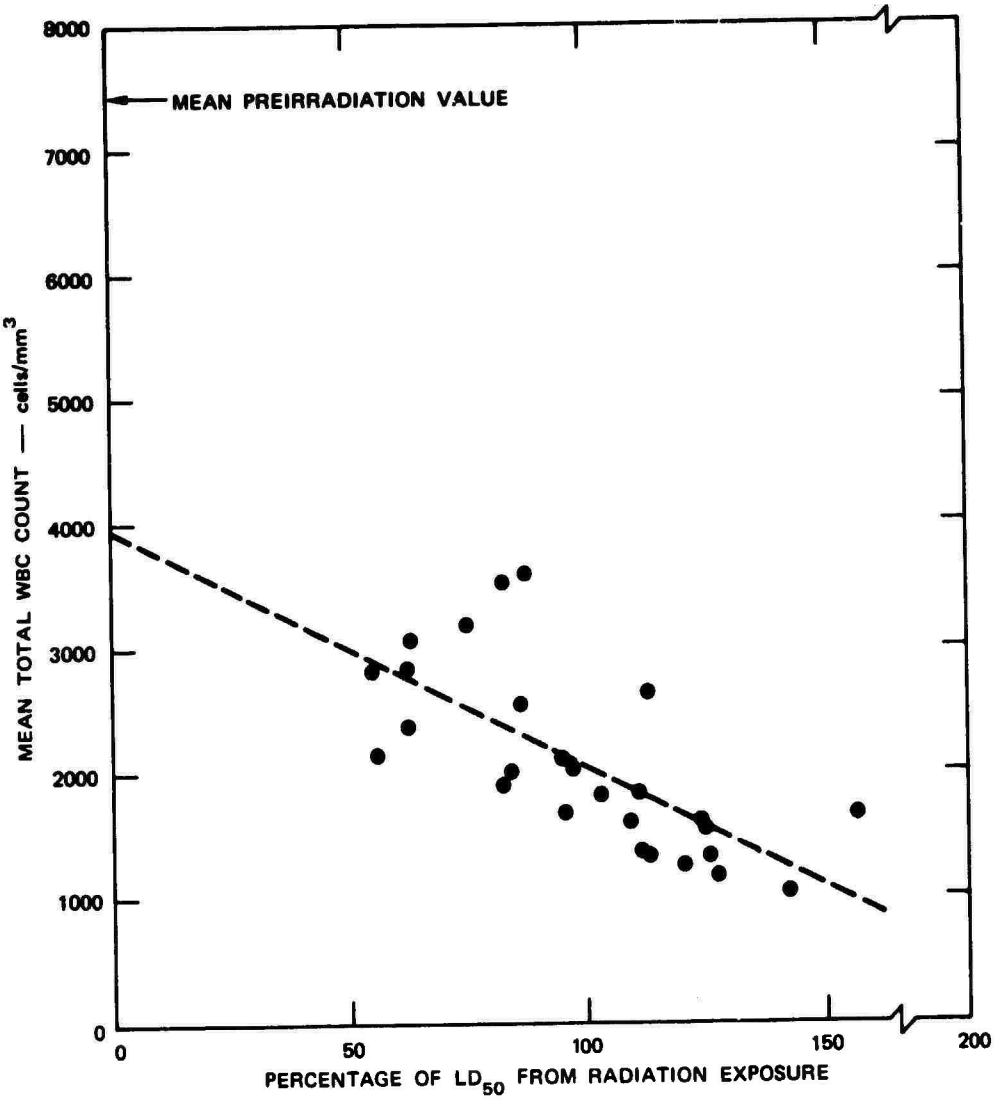

FIGURE 7 RELATIONSHIP OF TOTAL WBC COUNT TO RADIATION DOSE ONE WEEK AFTER COMPLETING CHRONIC RADIATION EXPOSURE

The pattern of total WBC count for the eight weeks following exposure to chronic irradiation is shown in Figure 8. The vertical bars on each point in the figure represent the 90% confidence intervals of the mean cell count, and nonoverlap of the bars implies that the probability of the differences occurring by chance is less than 1%. At all times from one through five weeks after completion of the radiation, the total WBC count of the survivors was greater than that of nonsurvivors. Nonsurvivors had cell counts of about 1500/mm^3, and there was no evidence of recovery of the cell count. Survivors reached the lowest WBC count at the third week after completion of the irradiation, and from the third through the eighth week the count appeared to show a weak recovery, about 100 cells/mm^3/week.

The mean cell counts for total WBC, granulocytes, and monocytes in sheep during the eight-week period of observation after chronic irradiation are listed in Table 14. Week 0 in this table designates the preirradiation mean cell count. The "t for Diff." (column 8) is the calculated value of Student's t for the difference in mean counts between surviving and nonsurviving animals. Values of t in excess of 2.0 indicate that the observed differences have less than 5% probability of being the result of chance. In addition, the values of t give some approximate (nonlinear) estimate of the efficiency of discrimination between survivors and nonsurvivors; larger values of t indicate that the difference is more reliable. The data in the table show that at all times after irradiation except in the sixth week, the cell count for total WBC, monocytes, and granulocytes is significantly higher in survivors than in nonsurvivors. However, at all postirradiation weeks (except the sixth, where only four nonsurvivors are left) the value of t for the difference in means is consistently higher for the total WBC counts than for the granulocyte or monocyte counts. This result indicates that the difference in total WBC count is more reliable than the difference in monocyte or granulocyte count for assessing the degree of injury following chronic radiation exposure.

An interesting feature of the data is the fact that the total WBC count before irradiation was slightly higher in animals that subsequently survived than in animals that died. Comparison of granulocyte and

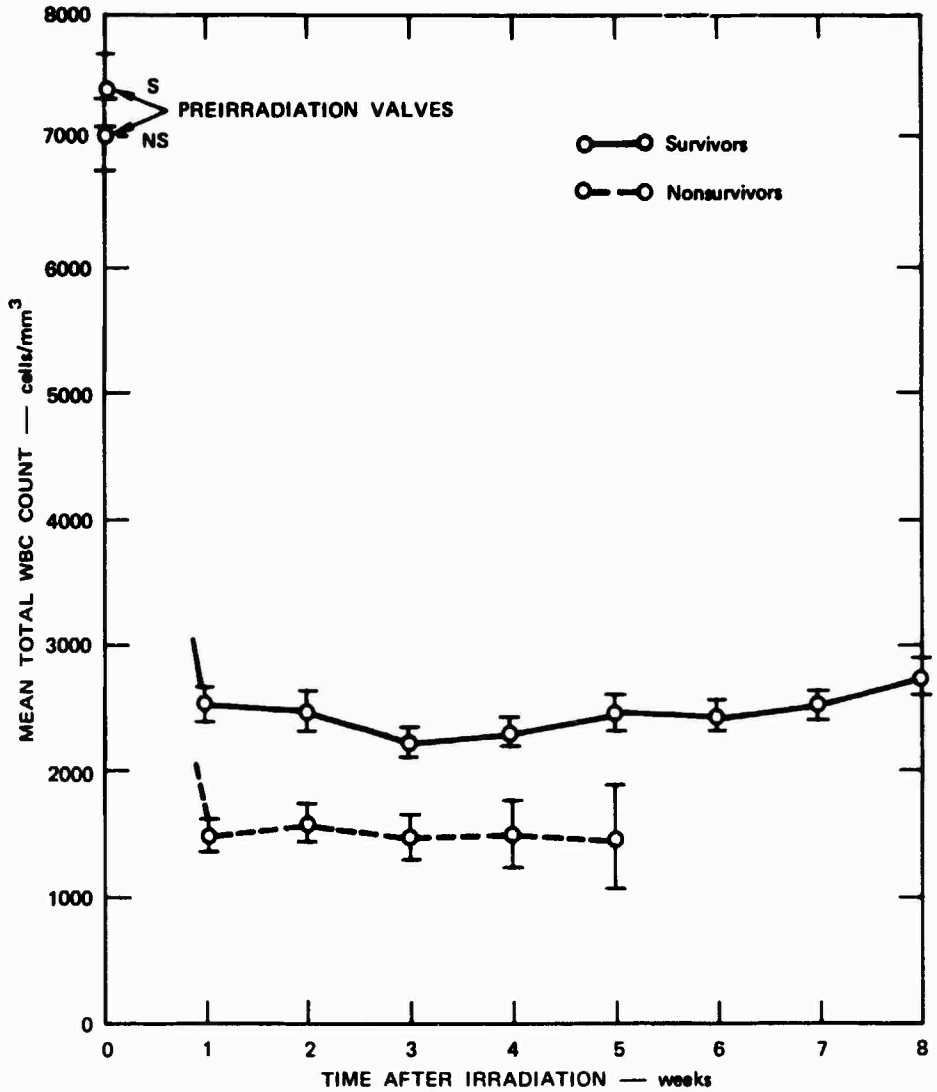

FIGURE 8 TOTAL WBC COUNT IN SHEEP DURING THE FIRST EIGHT WEEKS FOLLOWING CHRONIC EXPOSURE TO ^{60}Co GAMMA IRRADIATION AT TOTAL DOSES IN THE MIDLETHAL RANGE

Table 14

LEUCOCYTE COUNTS IN SHEEP DURING EIGHT WEEKS FOLLOWING COMPLETION OF CHRONIC EXPOSURE TO ^{60}CO GAMMA RADIATION AT DOSES IN THE MIDLETHAL RANGE (EXPERIMENTS 11, 13, 16, 18, 19, AND 20)

Week after Irrad.	Survivors			Nonsurvivors			t for Diff.
	Mean Count (cells/mm^3)	SD (cells/mm^3)	N	Mean Count (cells/mm^3)	SD (cells/mm^3)	N	
			Total WBC				
0	7,415	2,156	170	7,049	2,118	152	1.536
1	2,526	1,040	170	1,493	802	124	9.231
2	2,480	1,164	170	1,591	757	79	6.206
3	2,241	742	170	1,488	707	47	6.223
4	2,315	828	170	1,500	774	26	4.711
5	2,474	1,027	170	1,476	638	9	2.881
6	2,442	811	170	2,440	1,149	4	0.044
7	2,530	862	170	--	--	0	--
8	2,751	1,101	170	--	--		
			Granulocytic Cells				
0	1,455	1,030	170	1,446	964	152	0.079
1	488	454	170	178	193	124	7.129
2	496	620	170	139	152	79	5.046
3	297	272	170	75	71	47	5.548
4	301	302	170	60	74	26	4.042
5	365	297	170	131	182	9	2.340
6	388	372	170	540	621	4	0.797
7	409	384	170	--	--	0	--
8	485	468	170	--	--		
			Monocytic Cells				
0	5,967	2,076	170	5,504	1,888	152	2.087
1	2,032	880	170	1,315	762	124	7.280
2	2,018	982	170	1,451	717	79	4.596
3	1,944	635	170	1,412	701	47	4.962
4	2,014	727	170	1,439	756	26	3.724
5	2,109	967	170	1,345	508	9	2.347
6	2,054	645	170	1,900	559	4	0.472
7	2,122	710	170	--	--	0	--
8	2,266	884	170	--	--		

monocyte counts shows that the difference is entirely due to differences in the monocyte count. The preirradiation difference in monocyte count between survivors and nonsurvivors is significant at the 5% level, and the result suggests that under some circumstances, monocyte counts may serve to predict individual differences in resistance to the lethal injury caused by radiation.

The mean cell counts for total WBC, granulocytes, and monocytes in sheep during the eight-week period following exposure to acute radiation, A-C-A radiation, and acute reirradiation are shown in Tables 15, 16, and 17, respectively. Most of the generalizations applying to the cell count following chronic irradiation apply also to the other radiation schedules. The cell count is generally lower in nonsurvivors than in survivors, with a major exception being no significant difference between survivors and nonsurvivors at one week after acute irradiation. Again, the value of t for the difference between survivors and nonsurvivors is consistently higher for total WBC than for monocytes or granulocytes, suggesting that the monocyte and granulocyte counts offer no advantage over total WBC in assessing degree of lethal injury from ionizing radiation. Unlike the finding with chronic irradiation, the total WBC counts show no evidence of recovery during the three- to eight-week period following irradiation at the other schedules. For acute reirradiation of sheep previously exposed to chronic irradiation, the count of monocytic cells was significantly higher before irradiation in those sheep destined to survive, but this difference was not found in acute or A-C-A irradiation. Considering the results as a whole, it appears that there may be a real association between preirradiation monocyte count and survival potential of the animal, but the association may be restricted to selected schedules or circumstances of radiation exposure.

It was noted above that there was a weak dependence of WBC count on radiation dose at one week after irradiation. The question arises of whether or not the difference in cell counts between survivors and nonsurvivors noted in Tables 14 to 17 reflects the fact that the nonsurvivors had, on an average, larger radiation doses. To answer this, the linear regressions of mean WBC count on radiation dose were computed at one week

Table 15

LEUCOCYTE COUNTS IN SHEEP DURING EIGHT WEEKS FOLLOWING ACUTE EXPOSURE TO ^{60}CO GAMMA RADIATION AT DOSES IN THE MIDLETHAL RANGE (EXPERIMENTS 2, 3A, AND 7)

Week after Irrad.	Survivors			Nonsurvivors			t for Diff.
	Mean Count (cells/mm^3)	SD (cells/mm^3)	N	Mean Count (cells/mm^3)	SD (cells/mm^3)	N	
			Total WBC				
0	7,227	2,095	108	7,252	2,150	72	0.077
1	2,592	946	108	2,376	908	72	1.523
2	2,967	1,111	108	2,210	909	72	4.796
3	2,342	613	30	1,415	546	24	5.788
4	2,116	760	108	1,257	680	17	4.390
5	2,340	961	108	1,645	788	6	1.735
6	2,326	787	108	1,236	231	5	3.077
7	2,324	860	108	--	--	0	--
8	2,379	865	108	--	--		
			Granulocytic Cells				
0	2,353	1,358	108	2,210	1,212	72	0.722
1	1,121	607	108	981	496	72	1.632
2	1,601	889	108	1,078	758	72	4.097
3	515	305	30	218	251	24	3.836
4	606	578	108	218	384	17	2.669
5	784	698	108	231	263	6	1.924
6	722	522	108	180	260	5	2.300
7	711	480	108	--	--	0	--
8	751	453	108	--	--		
			Mo. ocytic Cells				
0	4,873	1,628	108	5,036	1,766	72	0.635
1	1,484	522	108	1,395	655	72	1.010
2	1,408	735	108	1,129	479	72	2.830
3	1,827	474	30	1,145	553	24	4.829
4	1,509	512	108	1,039	539	17	3.490
5	1,556	542	108	1,413	618	6	0.626
6	1,604	536	108	1,057	329	5	2.258
7	1,613	640	108	--	--	0	--
8	1,628	603	108	--	--		

Table 16

LEUCOCYTE COUNTS IN SHEEP DURING EIGHT WEEKS FOLLOWING ACUTE-CHRONIC-ACUTE (A-C-A) EXPOSURE TO ^{60}CO GAMMA RADIATION AT DOSES IN THE MIDLETHAL RANGE (EXPERIMENTS 1, 3, AND 6)

Week after Irrad.	Survivors			Nonsurvivors			t for Diff.
	Mean Count (cells/mm^3)	SD (cells/mm^3)	N	Mean Count (cells/mm^3)	SD (cells/mm^3)	N	
			Total WBC				
0	7,228	2,056	89	7,218	2,079	91	0.034
1	3,177	1,219	89	2,275	979	91	5.460
2	4,030	1,534	89	2,311	1,003	91	8.883
3	2,799	1,160	25	1,618	724	18	3.810
4	2,679	959	89	1,861	735	9	2.479
5	3,223	1,172	89	1,100	421	5	4.019
6	3,019	1,058	89	--	--	0	--
7	2,833	1,074	89	--	--		
8	2,837	975	89	--	--		
			Granulocytic Cells				
0	2,214	1,021	89	2,172	1,046	91	0.273
1	1,280	697	89	959	613	91	3.261
2	2,067	1,141	89	1,062	788	91	6.879
3	589	903	25	115	75	18	2.216
4	622	507	89	225	290	9	2.308
5	1,093	826	89	110	109	5	2.645
6	961	712	89	--	--	0	--
7	798	510	89	--	--		
8	758	381	89	--	--		
			Monocytic Cells				
0	5,026	1,628	89	5,030	1,912	91	0.016
1	1,913	858	89	1,321	561	91	5.463
2	1,913	722	89	1,252	509	91	7.093
3	2,209	879	25	1,506	691	18	2.819
4	2,058	776	89	1,493	514	9	2.130
5	2,130	778	89	989	338	5	3.246
6	2,059	825	89	--	--	0	--
7	2,035	863	89	--	--		
8	2,080	795	89	--	--		

Table 17

LEUCOCYTE COUNTS IN SHEEP DURING EIGHT WEEKS FOLLOWING ACUTE REEXPOSURE TO ^{60}CO GAMMA RADIATION AT DOSES IN THE MIDLETHAL RANGE OF ANIMALS SURVIVING PREVIOUS CHRONIC RADIATION EXPOSURE (EXPERIMENTS 5R, 8R, 11-12-13R, AND 16R)

Week after Irrad.	Survivors			Nonsurvivors			t for Diff.
	Mean Count (cells/mm^3)	SD (cells/mm^3)	N	Mean Count (cells/mm^3)	SD (cells/mm^3)	N	
			Total WBC				
0	3,783	1,201	75	3,452	1,398	115	1.687
1	2,410	997	75	1,822	808	115	4.443
2	2,893	1,333	75	1,928	1,113	115	7.391
3	2,056	610	75	1,569	847	90	4.159
4	2,025	649	75	1,420	725	46	4.744
5	2,051	653	75	1,305	561	23	4.945
6	2,092	752	75	--	--	0	--
7	2,043	592	75	--	--		
8	2,075	810	75	--	--		
			Granulocytic Cells				
0	586	418	75	764	598	115	2.245
1	589	437	75	473	384	115	1.919
2	684	528	75	397	441	115	4.060
3	247	172	75	138	173	90	4.051
4	203	160	75	88	108	46	4.303
5	236	197	75	81	63	23	3.700
6	201	165	75	--	--	0	--
7	241	229	75	--	--		
8	198	172	75	--	--		
			Monocytic Cells				
0	3,197	1,053	75	2,687	1,135	115	3.112
1	1,821	864	75	1,341	605	115	4.486
2	2,209	1,202	75	1,531	891	115	4.454
3	1,809	548	75	1,432	733	90	3.684
4	1,822	618	75	1,333	682	46	4.056
5	1,815	582	75	1,224	538	23	4.334
6	1,892	645	75	--	--	0	--
7	1,802	569	75	--	--		
8	1,876	761	75	--	--		

after irradiation for the individual chronic experiments (11, 13, 16, 18, 19, and 20), and all WBC counts at one week after irradiation in these experiments were corrected for dose to the expected values at the LD_{50} doses for the individual experiments. The means and standard deviations computed for these dose-corrected WBC counts were: survivors, 2373 ± 959 cells/mm^3; and nonsurvivors, 1783 ± 772 cells/mm^3. These values may be compared with those of Table 14: survivors, 2526 ± 1040 cells/mm^3; and nonsurvivors, 1493 ± 802 cells/mm^3. Thus, with dose-correction, the mean count in survivors was reduced, and the mean count in nonsurvivors was increased. However, the difference in mean cell count was still 590 cells/mm^3, and the t for the difference was 5.498, a highly significant value. It is concluded that the difference in mean total WBC count between survivors and nonsurvivors partly reflects differences in average dose received in the experiments, but that there is an additional difference reflecting sensitivity of the animals to the radiation, and the difference in sensitivity for total WBC count is partly correlated with difference in sensitivity to lethal effects of the radiation.

Prediction of Death and Survival of Irradiated Sheep from Leucocyte Counts

Since the mean WBC, granulocyte, and monocyte counts at week one after irradiation are different for survivors and nonsurvivors, it is of interest to determine whether or not the cell counts have any value in predicting the death or survival of individual animals. To test this, the value of total WBC count the first week after irradiation, which represented the most probable dividing point between survivors and nonsurvivors, was determined for the chronically irradiated sheep. The determination was made according to

$$\text{Count} = \bar{X}_1 + (\bar{X}_2 - \bar{X}_1) \frac{SD_1}{SD_1 + SD_2} \qquad (1)$$

where $\bar{X}_1$, SD_1, $\bar{X}_2$, and SD_2 refer to the mean counts and standard deviations of the nonsurvivors and survivors, respectively. For the

chronically irradiated animals, the value of the total WBC count was 1941 cells/mm^3, about 26% of the mean preirradiation value. A similar dividing point was determined for granulocytes and monocytes at one week after irradiation, and the respective values were 270 and 1648 cells/mm^3. WBC, granulocyte, and monocyte counts of all of the 293 sheep in the chronic irradiation group were individually compared with the above cell counts, and animals with counts at one week after irradiation that were above the dividing points were predicted to survive, while animals with counts below the dividing points were predicted to die. The life/death evaluations were made separately for total WBC, granulocyte, and monocyte counts.

Table 18 shows the results of the predictions. Of 293 sheep, 150 were predicted to die on the basis of WBC count, 124 actually died, and of those that died, 98 sheep, or 79%, were correctly predicted. Similarly, 143 sheep were predicted to survive, 169 actually survived, and of those that survived, 116, or 69%, were correctly predicted. The overall number of correct predictions for total WBC was 214 out of a possible 293, or 73% of the total. Similar and comparable results were obtained by using granulocyte or monocyte counts instead of total WBC, but the percentage of correct predictions is no higher with these counts than with total WBC. The granulocyte and monocyte counts offer no advantage over total WBC in predicting animal survival.

A similar analysis was made of the prediction of death or survival in other irradiated sheep, using WBC counts only, and the same dividing point (1941 cells/mm^3) as was used above for chronically irradiated animals. The results are shown in Table 19. The chronic irradiation animals (column 1) are the animals from other experiments (e.g., 5 and 8), which were not used in computing the WBC counts for chronic animals in Table 15 because the doses used were too low or too high to give a balanced mortality. This group also included Experiment 22, which involved periodic irradiation at a high dose rate, rather than a low dose rate.

Table 18

PREDICTION OF SURVIVAL OF SHEEP FROM BLOOD LEUCOCYTE COUNTS ONE WEEK AFTER COMPLETION OF CHRONIC EXPOSURE TO ^{60}CO GAMMA RADIATION AT DOSES IN THE MIDLETHAL RANGE (EXPERIMENTS 11, 13, 16, 18, 19, AND 20)

	Prediction by		
	Total WBC Count	Granulocyte Count	Monocyte Count
Total number of animals	293	293	293
Number predicted to die	150	161	161
Number dying	124	124	124
Number of correct predictions	98	101	98
Percent correctly predicted to die	79.0%	81.5%	79.0%
Number predicted to survive	143	132	132
Number surviving	169	169	169
Number of correct predictions	116	109	105
Percent correctly predicted to survive	68.6%	64.5%	62.1%
Total number of correct predictions	214	210	203
Percent of total number correctly predicted	73.0%	71.7%	69.3%

Table 19

PREDICTION OF SURVIVAL OF SHEEP FROM BLOOD TOTAL WBC COUNTS ONE WEEK AFTER COMPLETION OF EXPOSURE TO ^{60}CO GAMMA RADIATION IN VARIOUS TYPES OF EXPOSURE SCHEDULES

	Exposure Schedule			
	Chronic*	Acute	A-C-A	Reirradiated
Total number of animals	201	180	180	188
Number predicted to die	74	49	49	95
Number dying	72	71	91	113
Number of correct predictions	46	28	38	72
Percent correctly predicted to die	63.9%	39.4%	41.8%	63.7%
Number predicted to survive	127	131	131	93
Number surviving	129	109	89	75
Number of correct predictions	101	91	79	52
Percent correctly predicted to survive	78.3%	83.5%	88.8%	69.3%
Total number of correct predictions	147	119	117	124
Percent of total number correctly predicted	73.1%	66.1%	65.0%	66.0%

*Experiments 5, 8, 21, 22, and 23.

For the chronic irradiation, the overall percentage of correct predictions was the same as that shown in Table 18 for the other chronic irradiation series. For the acute, A-C-A, and reirradiated series, the fate of approximately two-thirds of the irradiated animals can be determined from the total WBC count at one week after irradiation, even when the dividing cell count was standardized for another schedule of irradiation. The results in Table 19 show that the prediciton of survival is generally better than the prediction of death. This bias may be the result of the fact that the dividing cell count was based on the data for a selected set of chronic irradiation experiments, rather than the experiments analyzed in the table.

Prediction of Death from Very Low Granulocyte Counts

Following irradiation, both the granulocyte and monocyte counts are reduced, and remain in a depressed state. Granulocyte counts, in particular, can fall to levels of virtually zero in individual animals during the postirradiation period. An investigation was made of whether these low counts had any significance as an indicator of probable or imminent death. The data of both surviving and nonsurviving animals were scanned, and the incidence was tabulated of granulocytic counts of less than 100 cells/mm^3 at any time following irradiation.

The results of the survey are shown in Table 20. In the acute and A-C-A experiments, the finding of a low granolocyte count in an animal indicated that the animal was four times as likely to die as to survive, but most of the animals that died did not show such low granulocyte counts. In the chronic and reirradiated experiments, a large fraction of both survivors and nonsurvivors showed low granulocyte counts, and no special likelihood of death can be inferred from low granulocyte counts in these animals. The general incidence of low granulocyte counts is consistent with the mean counts shown in Tables 14 to 17. It is concluded that low granulocyte count has no special value in the prognosis of radiation sickness in sheep.

Table 20

INCIDENCE OF VERY LOW COUNTS (< 100/mm^3) OF GRANULOCYTIC CELLS IN SURVIVING AND NONSURVIVING SHEEP EXPOSED TO VARIOUS SCHEDULES OF IONIZING RADIATION

Irradiation Schedule	Status	Number of Animals	Number with One Or More Low Granulocyte Counts	Number of Postirradiation Measurements	Number of Low Granulocyte Counts
Acute	Surviving	108	6	786	7
	Nonsurviving	72	24	196	27
A-C-A	Surviving	88	5	641	6
	Nonsurviving	92	19	218	22
Chronic*	Surviving	101	61	898	101
	Nonsurviving	64	51	151	75
Reirradiated	Surviving	75	56	600	128
	Nonsurviving	115	85	394	136

*Experiments 11, 13, and 16.

Erythrocyte (RBC) Counts and Packed Cell Volumes (Hematocrits)

RBC counts and hematocrits were measured on all blood samples taken. As noted earlier in this chapter, the variance of the RBC count is much smaller than that of WBC. Paradoxically, however, the response of RBC count to irradiation appears to be more erratic than that of WBC count. Figure 9 shows the mean RBC count during and after continuous or periodic exposure to radiation for Experiments 5, 11, 13, and 16. The RBC count, on the ordinate, is represented as the percentage of the preirradiation value, and each point represents a mean of 9 to 12 animals. The solid points, on the left side of the figure, represent the period during which radiation was administered, the open points on the right represent the postirradiation period, and the dashed lines show the transition from irradiation to postirradiation period.

The results varied. In three experiments, there were periods during irradiation where the mean RBC count was greater than preirradiation values. In one experiment, the RBC count rose from 82 to 108% of the control value during the first three weeks after irradiation. In another experiment, the RBC count during irradiation fell precipitously; in the others, it oscillated around 100% of the control value. The most stable period for all RBC counts was the period from five to eight weeks after irradiation, where the mean count ranged from 75 to 90% of the preirradiation control value.

RBC counts following acute and A-C-A irradiation and reirradiation, of course, did not have values during irradiation. In the postirradiation period, the mean RBC counts fluctuated irregularly during the first four weeks, and then stabilized in the region of 70 to 90% of control pre-irradiation values during the following four weeks.

The mean RBC counts during the period from five to eight weeks after irradiation had a weak relationship to radiation dose. Figure 10 shows the relationship of mean RBC count averaged over the fifth through the eighth week to radiation dose. RBC counts are expressed as percentage of control preirradiation values, and the radiation dose is expressed as percentage of the LD_{50}. The open circles in the figure are from acute

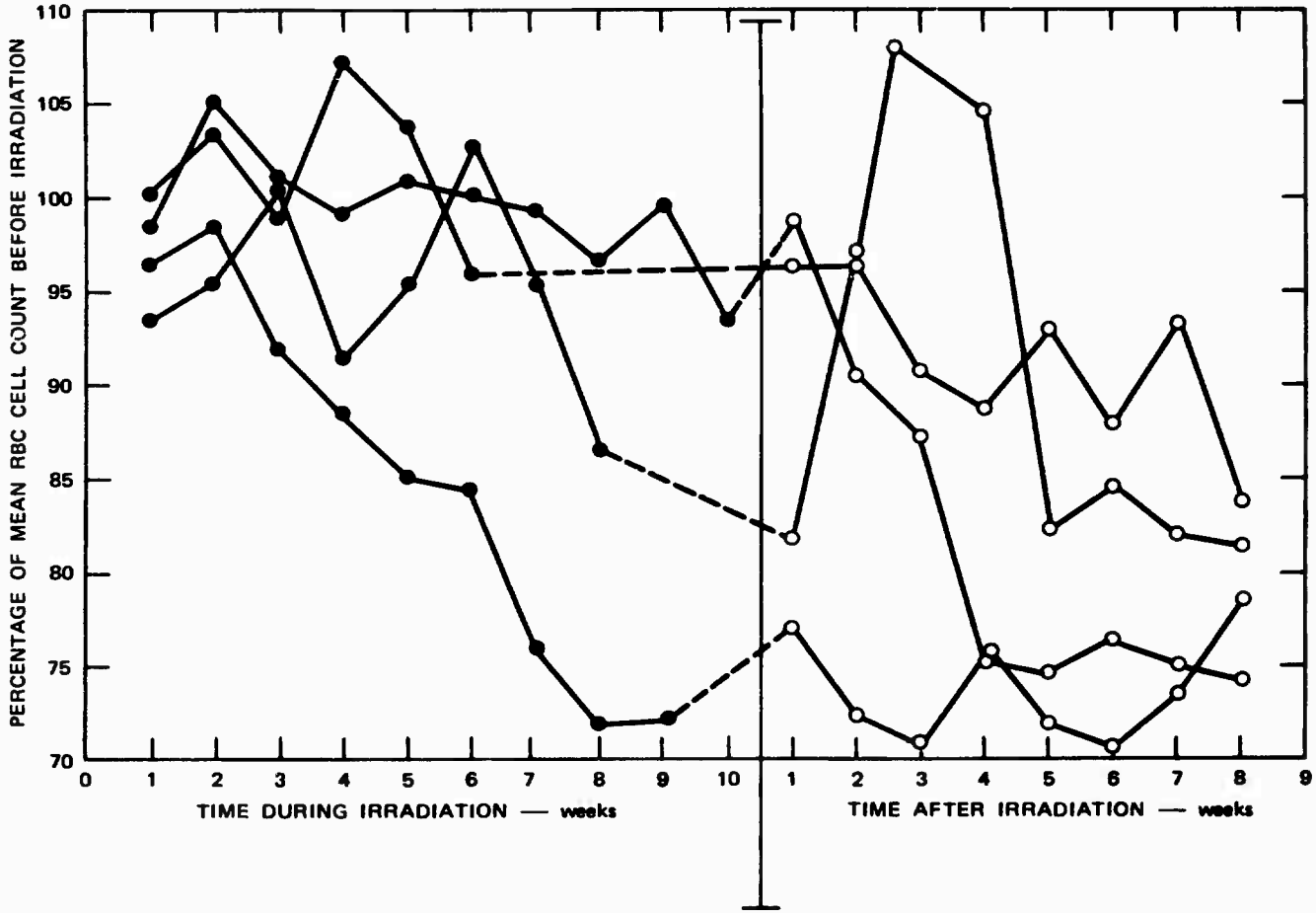

FIGURE 9 MEAN RBC COUNTS IN SHEEP DURING AND FOLLOWING EXPOSURE TO CONTINUOUS OR PERIODIC RADIATION WITH ^{60}Co GAMMA RAYS AT LOW DOSE RATE

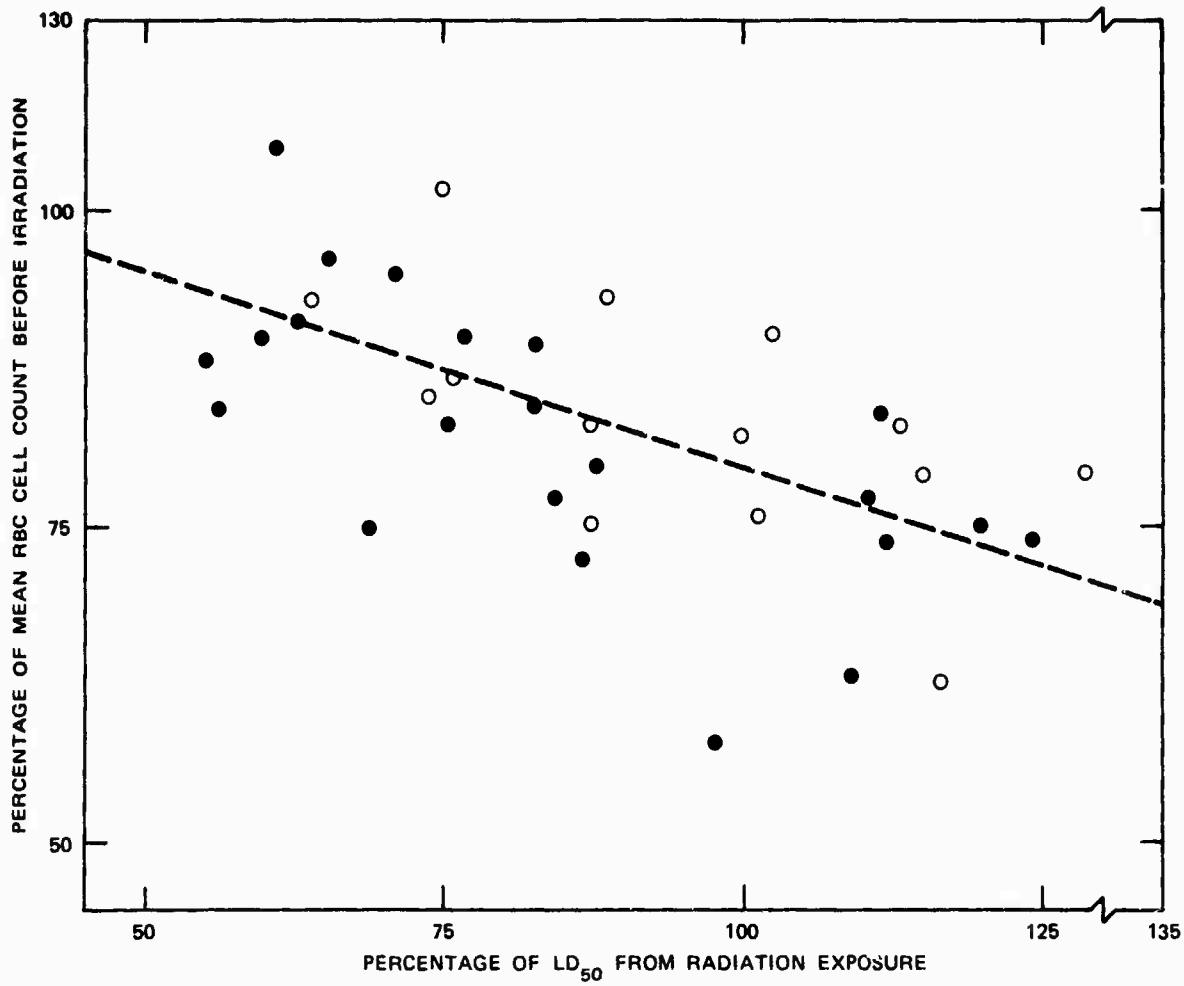

FIGURE 10 DEPENDENCE OF MEAN RBC COUNT ON RADIATION DOSE IN THE FIVE TO EIGHT WEEK PERIOD FOLLCWING EXPOSURE TO ^{60}Co GAMMA RADIATION

experiments (2, 3A, and 7), and the solid dots are from chronic or periodic low dose-rate experiments (5, 11, 13, and 16). The RBC counts for the acute experiments were slightly, but not conspicuously, higher than those of the chronic and periodic experiments. The dashed line in the figure represents the linear regression of RBC count on radiation dose. The slope of the line is - 0.309% of RBC count for each percentage of LD_{50}, and the line reaches 100% of the RBC count at 34.3% of the LD_{50}.

The individual values of RBC count in the period during or following irradiation ranged at the extreme down to as low as 20% of preirradiation values. Although clinical examinations were not made, we assume that low RBC counts were the result of hemorrhage, and we made a survey of the data to determine whether hemorrhage might be associated with death of the animals. For purposes of definition, we arbitrarily selected an RBC count of 50% of the preirradiation value as presumptive evidence of hemorrhage, and we tabulated the occurrence of such levels at least one time during the period of observation for several experiments, and noted whether the animals survived or died.

The results of the survey are presented in Table 21. Of 345 animals, 136 died, and 8 of those dying, or 6%, showed very low RBC counts; the rest did not. Among the 209 surviving animals, 16 sheep, or slightly less than 8%, showed very low RBC counts. It is concluded that hemorrhage is not a significant cause of death in sheep, and that evidence of hemorrhage does not have prognostic value for judging likelihood of death or survival.

Hemocrits (packed cell volumes) generally showed values that were parallel to those of RBC count. The average RBC:PCV ratio in unirradiated sheep was 30.17 X 10^6 packed cells/mm^3, giving a mean cell volume of 33.1 μ^3. The values of RBC:PCV ratio ranged at the extremes from 24 to 36 X 10^6 packed cells/mm^3, and the ratio was normally distributed. Although we prefer using total RBC count for analysis, the evidence indicates that PCV could be substituted for RBC count without loss of essential information.

Table 21

INCIDENCE OF LOW RBC COUNTS (≤ 50% OF CONTROL) AMONG SURVIVING AND NONSURVIVING SHEEP EXPOSED TO MIDLETHAL DOSES OF ^{60}CO GAMMA RAYS

Experiment Number	Number of Animals	Number Dying			Number Surviving		
		Total	With Low RBC	Without Low RBC	Total	With Low RBC	Without Low RBC
11	60	27	4	23	33	5	28
13	35	14	1	13	21	0	21
16	70	23	1	22	47	3	44
2	60	21	0	21	39	2	37
3A	60	30	1	29	30	2	28
7	60	21	1	20	39	4	35
Totals	345	136	8	128	209	16	193

In connection with the study of RBC:PCV ratios, an extensive survey was made of the ratio during the postirradiation period in chronically irradiated animals to determine whether irradiation resulted in significant changes in the average size of the RBCs. With a single exception (Experiment 8), the mean RBC:PCV ratio after irradiation was 30.03, the same value as was found for unirradiated sheep. In Experiment 8, the mean RBC:PCV ratio was 26.65, giving a mean cell volume of 37.5μ^3. In the absence of similar findings in the other experiments, it is concluded that the result in Experiment 8 was probably the result of some incidental event not associated with the radiation.

Long-Term Recovery of Blood Cell Counts

Two groups of survivors from early radiation experiments were saved for study of long term hematological recovery. One group, from Experiment 7, had received 193 R at 561 R/hr (acute radiation), which represented 64% of the estimated LD_{50} for the experiment. The other group, from Experiment 5, had received 925 R at 0.9 R/hr (chronic, low dose rate irradiation), which represented 83% of the LD_{50}. The 60-day mortality was 1/12 and 2/12 for the two groups. Blood samples were taken, and hematological analyses were performed at intervals of approximately four weeks for over a year after completion of the irradiation.

The course of recovery of total WBC counts is shown in Figure 11. The open circles in the figure represent the acutely irradiated animals, and the solid points represent the chronically irradiated animals. Both groups started at a WBC count of about 2550 cells/mm^3, and the course of recovery was essentially the same in both. The WBC count rose slowly, and, except for an upward excursion during weeks 45 to 50, appeared to be stabilizing at a level of 5500-6000 cells/mm^3--a level of 70 to 76% of the original preirradiation count. The proportion of granulocytic and monocytic cells remained approximately constant during the period of observations. The mean RBC counts in the period from 30 to 62 weeks after irradiation were 94.4 and 89.5% of the preirradiation values for the acute and chronic experiments, respectively, and these values were not significantly different from those found in the 5- to 8-week period after irradiation.

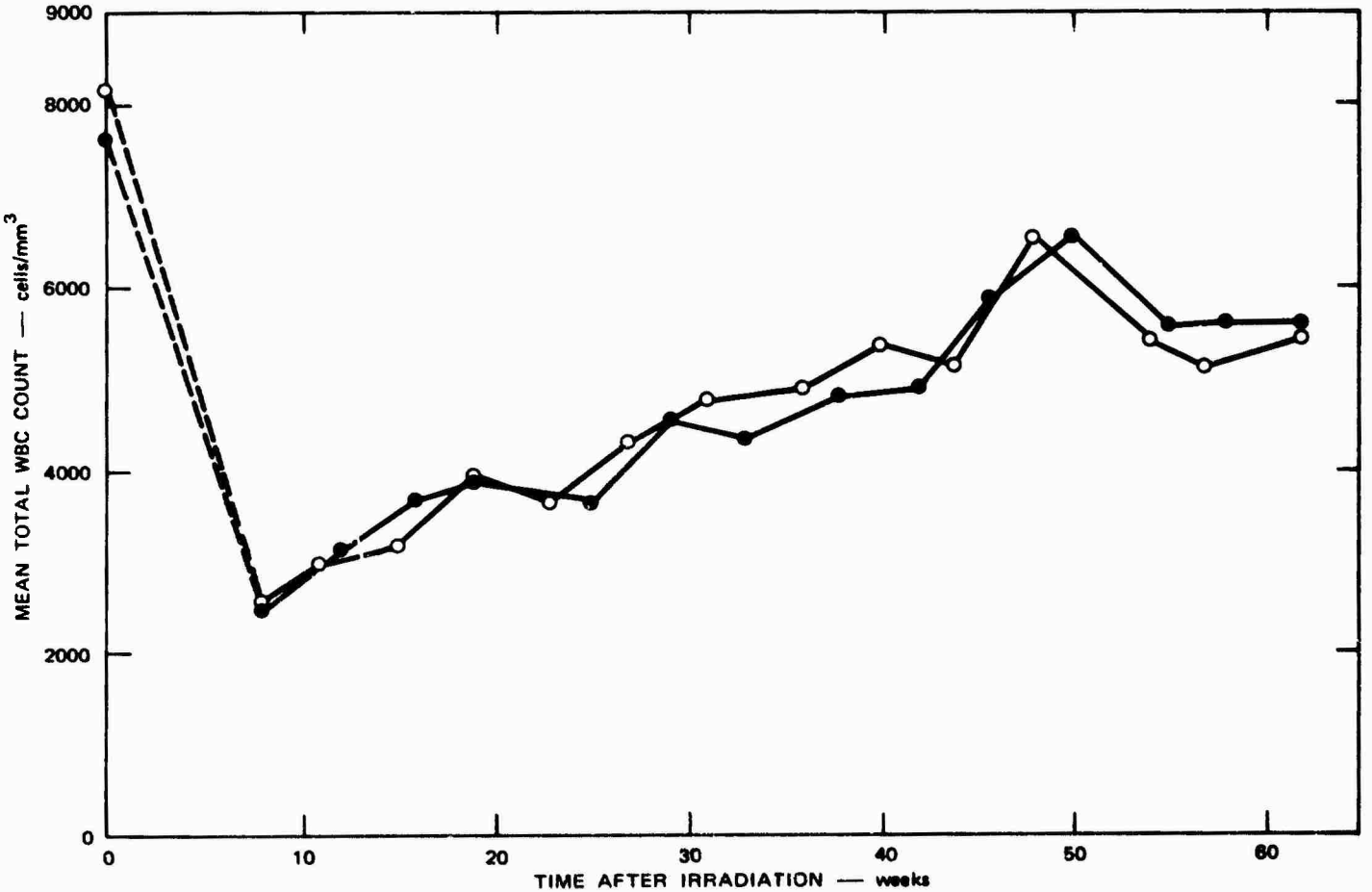

FIGURE 11 LONG-TERM RECOVERY OF TOTAL WBC COUNT IN SHEEP EXPOSED TO MARGINALLY LETHAL DOSES OF ^{60}Co GAMMA RAYS AT LOW EDR (SOLID POINTS) OR HIGH EDR (OPEN CIRCLES)

It is concluded that the recovery of total WBC count after irradiation is slow, and probably never complete. Granulocytic and monocytic cells have about the same potential for recovery, and RBC counts remain fixed at stable postirradiation values, except possibly for recovery from nonfatal hemorrhage.

Summary and Conclusions

The data presented here show that the most consistent and reliable hematological indicator of lethal radiation injury is the total WBC count. Counts of granulocytic and monocytic cells, under certain conditions, may also indicate the level of lethal injury, but, primarily because of their greater variability, these cell counts do not offer any advantage over total WBC counts. RBC counts show a depression that is proportional to the level of lethal injury, but the count depression does not become stable until most of the deaths from radiation injury have already occurred.

The mean total WBC count in sheep at one week after completion of midlethal radiation exposure ranged from around 1500 to around 3000 cells/mm^3 in various experiments. Animals destined to survive had higher mean WBC counts, while animals that subsequently died had lower mean WBC counts. Based on a dividing WBC count of 1941 cells/mm^3 (26% of mean preirradiation value), animals could be predicted to live or die according to whether the WBC count was above or below the dividing score, and the predictions were correct between 66 and 73% of the time. Very low granulocyte counts were only marginally useful in predicting death of animals, and low RBC counts were of no value at all.

It should be pointed out that the findings and conclusions presented in this chapter are based on what might be called "field" conditions of operation. Animals were sampled at regular intervals after exposure, but no attempt was made either to treat or to follow up animals with low counts or overt signs of severe illness. Hence, incidences of fatal hemorrhage or precipitous drop in WBC, granulocyte, or monocyte counts could have occurred just before death without being detected by the

sampling procedure. The blood sampling schedules used in this study are somewhat comparable to what might have to be accepted in a public emergency where a nuclear disaster involved widespread deposition of radioactive material in a populated area. The conclusions concerning the value and utility of hematological observations in the present study would probably apply equally well tc the conditions of an extensive nuclear disaster.

IV CELLULAR CHANGES IN BONE MARROW OF MICE AND SHEEP DURING AND AFTER EXPOSURE TO ^{60}CO GAMMA RAYS

The cell systems in the adult mammal that are most vulnerable to the acutely lethal effects of ionizing radiation have been known since the earliest studies of biological effects of ionizing radiation. These systems are those that are in a nearly continuous state of renewal and cell proliferation: epidermis, gastrointestinal epithelium, bone marrow, lymph nodes, and gonads. Whole-body irradiation of mammals at lethal doses will result in extensive destruction and depletion of cells from all of these systems, but the cell system most regularly associated with death of animals from irradiation is the bone marrow.

Despite the early identification of the bone marrow as the principal site of lethal injury, quantitative studies to link cellular events in the marrow with death of the animal are few in number and incomplete in definition. The main reasons for this deficiency are technological in nature. Bone marrow contains several developing cell lines, each with a number of cell types or stages of development, and identification and correct assignment of cells observed in a marrow specimen are time-consuming and difficult. In addition, marrow is not contained in a discrete organ, but is distributed nonuniformly over most of the skeleton, so that quantitative estimates of the total number of marrow cells lost and replaced after irradiation are difficult and problematic in accuracy. Finally, although a number of elegant methods have been developed to study cell viability and replacement in vivo, many of these methods are restricted to rats and mice, in which the ionizing radiation LD_{50} is higher than in many large animal species, including man.

This project has been mainly concerned with the relationship of radiation dose rate to the lethal effects of gamma rays in large animals using the sheep as an experimental animal. The limited number of bone marrow studies conducted have been directed to questions of the mechanism

of dose rate effects at the level of the marrow cell and the relationship of marrow cell counts to cell counts of formed elements of the blood. The studies are neither exhaustive nor comprehensive, but they indicate some of the cell mechanisms that may be involved in the relationships between dose rate and lethality considered in other chapters of this report.

Relationship among Bone Marrow Colony-Forming Cells, Dose Rate, and LD_{50} in Mice

This study involved comparison of the degree of depletion of bone marrow colony-forming cells with the lethal effects of ^{60}Co gamma rays in mice, when the gamma rays were given at two different dose rates. The bone marrow colony-forming cells were estimated by the procedure of Till and McCulloch,[12] the relative lethal effects were estimated by determination of the single-exposure LD_{50}, and the dose rates were 1750 and 190 R/hr. The details of the study have been published in the open literature,[13] and the results and conclusions are summarized here only briefly.

The LD_{50}s were 873 R at 1750 R/hr and 1359 R at 190 R/hr. The ratio was 1.56, indicating that irradiation at the higher dose rate was over 50% more effective in causing death than irradiation at the lower dose rate. Despite this difference in LD_{50}, the depletion of colony-forming cells from the bone marrow was the same at both dose rates. Depletion of the cells is customarily represented by the formula

$$S/S_o = Ke^{-D/D_o} \qquad (2)$$

where S/S_o is the surviving fraction of cells after radiation dose D, and K and D_o are constants, usually referred to as the extrapolation number and the D_{37}, or 37% survival dose. In measurements out to a total radiation dose of 400 R, the values of K were 1.93 and 1.83, and the values of D_o were 90.1 and 92.9 R, for dose rates of 1750 and 190 R/hr, respectively. Thus, the survival, or depletion, of colony-forming cells of the bone marrow was essentially the same at both dose rates, and the difference

in lethal efficiency of the radiation could not be accounted for by a difference in the survival of these bone marrow cells at the two dose rates. The colony-forming cells of the marrow represent at least a fairly early stage in the development of marrow elements, and would be expected to play a significant role in the regeneration of marrow after irradiation.

The highest dose used in the studies on the survival of bone marrow colony-forming cells was 500 R, and the mean survival fraction at this dose was of the order of 8×10^{-3}. This level of survival fraction is very nearly the practical lower limit for assay by the method, and hence studies of cell survival cannot be extended much beyond 500 R. It is possible that at higher doses, the survival curve of the colony-forming cells bends and proceeds at a shallower slope (higher value of D_0), and that the degree of change of slope is related inversely to dose rate. This would imply that if effects of dose rate on mortality depend on effects of dose rate on cell survival, then the effects occur in a region of dose and cell survival where the proposition cannot be tested.

Attention is directed at this time to another matter, which will be discussed subsequently. At the dose rate of 1750 R/hr, the time required to deliver the maximum dose of 500 R was about 17 min, while at the dose rate of 190 R/hr, it was about 2 hr, 38 min. Thus, if dose-rate effects do exist for cell survival, they do not depend on duration of exposure out to a period of 2-1/2 hr, even though dose-rate effects on LD_{50} do exist in several species when the duration of exposure exceeds 1/2 hr (see Chapter V).

Effect of Dose Rate on the Total Bone Marrow Cell Count in Mice Following Exposure to Ionizing Radiation

A method was developed for estimating the total bone marrow cell count in animals. The method was originally developed in mice, and further studies showed that it could also be applied to sheep. The procedures and the validity of the method are described in detail in Appendix A of this report.

For studies in mice, the animals were exposed to 500 R at either 1550 R/hr or 155 R/hr. The mice were LAF_1 females approximately 8 to 11 weeks old at the time of the study. Total bone marrow cell count was assessed at 2, 4, 7, 8, 11, and 16 days after irradiation. The studies were done in two sets of experiments approximately one year apart. There was no significant difference between the results in the two sets of experiments, and the results of the two sets were combined.

The results of the study are shown in Figure 12. Solid points in the figure represent total bone marrow cell count following 500 R at 155 R/hr; open circles represent total bone marrow cell count following 500 R at 1550 R/hr. The vertical bars on each point represent the 90% confidence intervals for the mean of ten pairs of mice used in each measurement. The mean total bone marrow cell count in unirradiated mice was 1.021×10^9 cells, with a 90% confidence interval of $0.937 - 1.106 \times 10^9$. This value is represented by a horizontal band across the figure. The mean total bone marrow cell count at the moment when irradiation was complete cannot be estimated because of the nature of the test. However, if the survival of all bone marrow cells is assumed to be the same as that of colony-forming cells, then the total surviving bone marrow cell count would be 8.13×10^6 for exposure at either dose rate. This value is represented by another horizontal line on the figure.

Inspection of the figure shows that the recovery of bone marrow cell count after irradiation involves several stages. From about days one to five, there is rapid replacement of the total marrow cell count; from about days five to eight, there is a plateau region at around or just below the normal marrow cell count; at day 11, the marrow cell count is significantly above (about twice) the normal level; finally, at day 16, the bone marrow cell count has returned to the normal level. Except for the first stage, there appears to be little difference in the process of marrow replacement at the two dose rates.

In the first stage of recovery, the marrow cell count increased from 0.15×10^8 to 2.16×10^8 cells after irradiation at 1550 R/hr, and from 0.42×10^8 to 6.56×10^8 after irradiation at 155 R/hr. The increase,

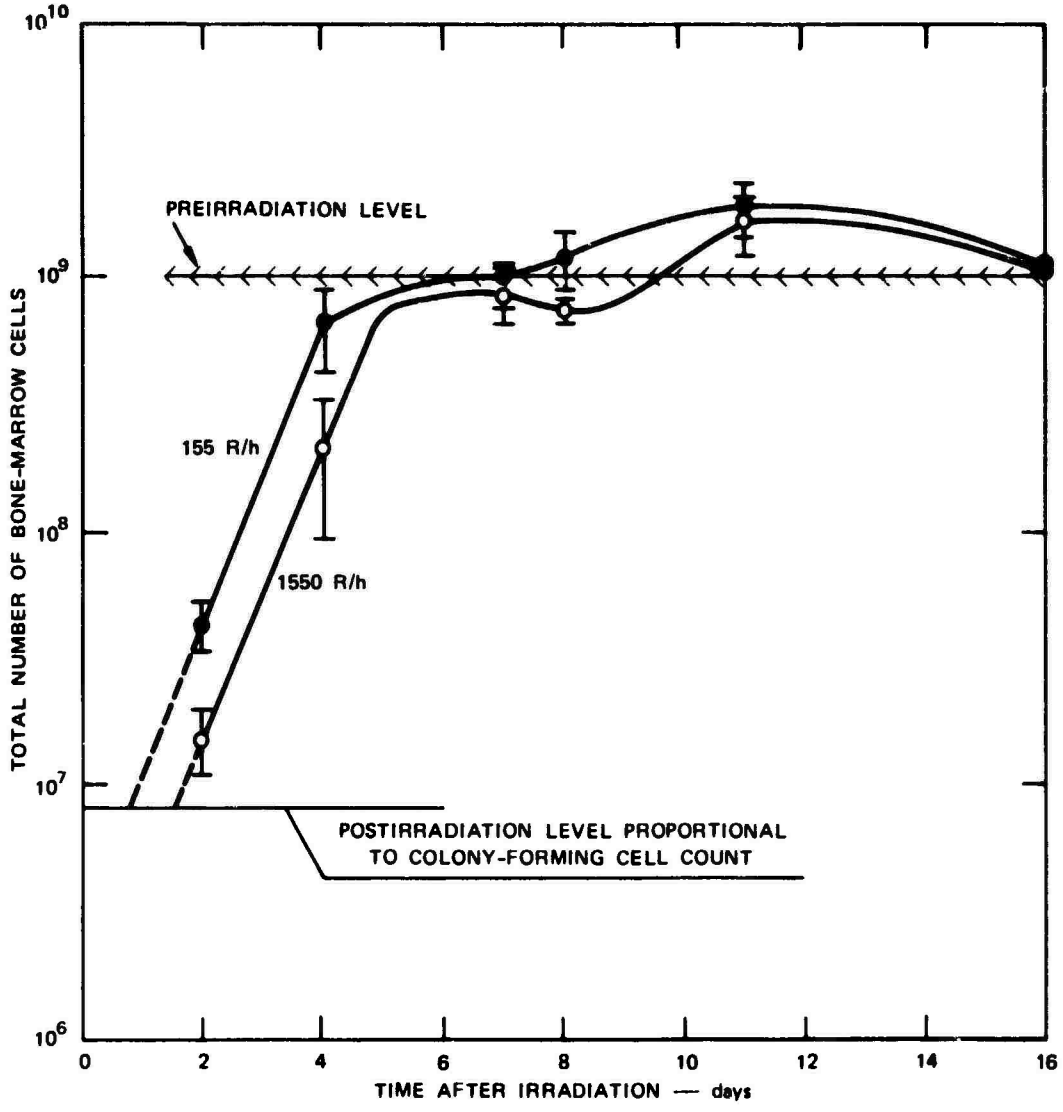

FIGURE 12 TOTAL BONE MARROW CELL COUNT IN MICE AFTER EXPOSURE TO 500 R OF ^{60}Co GAMMA RAYS

of about fifteen-fold in two days, was the same proportion for both dose rates, but the recovery after 155 R/hr is clearly more advanced than after 1550 R/hr. The increase between days two and four represents a cell doubling time of 12.1 to 12.5 hr. This time is approximately the time required for each mitotic cycle, and the result implies that during this time period the replacement of bone marrow cells is proceeding at the maximum possible rate.

By extrapolating the lines for recovery between days two and four back to the expected surviving bone marrow cell count at the time when irradiation was complete, it was found that the replacement of the marrow began at 18.8 hr after irradiation at the 155 R/hr dose rate and 37.1 hr after irradiation at the 1550 R/hr dose rate. These times do not, of course, represent the exact times of commencement of the marrow recovery, but they do represent the difference in recovery for the two dose rates. Following irradiation, one might expect mitotic delays in surviving proliferative cells, autolysis of dead cells, some disorganization of the structure of the marrow, and possibly some further loss of marrow cellularity from secondary effects of the radiation. By the second day after irradiation, recovery of marrow cellularity is proceeding at the maximum rate, and the degree of recovery at this time is as if recovery began 18.3 hr earlier for the lower dose rate.

It is concluded that irradiation at the lower dose rate has a dose-sparing effect on bone marrow, not because the destruction of marrow cells is less, nor because rate of recovery of marrow cellularity is greater, but because the recovery of marrow cellularity begins sooner after the irradiation at a lower dose rate. The dose equivalence of the earlier recovery can be calculated as follows: The 18.3-hr time difference applies to a proliferating cell system with a mean doubling time of 12.3 hr; the equivalent gain in cellularity of the marrow is a factor of 2.80. This implies that the marrow cell recovery after 1550 R/hr proceeds as if it had started at the same time as after 155 R/hr, but at a level 2.80 times lower. The mean value of D_o for the colony-forming cells was 91.5 R. If this value is assumed to hold for the bone marrow cells in general,

then the dose equivalent to 2.80 times reduction in cell count is given by the equation

$$\frac{1}{2.80} = e^{-\frac{D}{91.5}} \qquad (3)$$

The equivalent value of D is 94 R, about 19% of the total dose given.

Total Bone Marrow Cell Count in Sheep Following Exposure to ^{60}Co Gamma Rays

It was noted in the introduction to this chapter that, in the evaluation of the relationship of lethal effects of radiation to its effects on bone marrow, two of the major problems are the identification of marrow cells of the various lines and stages of development and the quantitative estimation of the total number of marrow cells. Attempts were made to attack both problems. The first problem was approached by attempting to apply a staining procedure that would identify cells of the erythroid line; this approach was abandoned because preliminary studies indicated that the method was unreliable and time-consuming. The second problem was approached by using a ^{59}Fe-labeling procedure for marrow similar to that for mice; this method (Appendix A) was rendered successful and allowed for some interpretation of radiation effects on marrow in the sheep, even though the variance of the results is greater than desirable.

Studies were carried out in unirradiated sheep and in sheep exposed to radiation at various values and combinations of EDR and ADR. Because of cost, only a relatively few experiments were conducted for the sake of marrow studies alone, and these experiments were done at the beginning of the series, when details of the procedure were being developed. Most of the bone marrow experiments were conducted as adjuncts to other radiation experiments, where the principal objective was lethality studies.

Studies were conducted in 108 sheep. Of this total, 8 were discarded because of a technical error in the assay procedure, and 4 others were discarded because a marrow specimen could not be obtained. Of the remaining 96 sheep, in which total bone marrow cell count could be estimated

5 additional animals were discarded because their total marrow cell counts were grossly too large. The most reasonable explanation for the exceptionally large counts is that the marrow cells in these specimens were not diluted and counted sufficiently soon after removal from the animal. Autolysis of the cells in the sample leads to spuriously high total marrow count values.

The decision to eliminate these 5 animals was based not on the effect of elimination on the mean values of the group they were in, but by the effect on the overall variance of the groups. The pooled standard deviation for all of the groups, with all 96 animals included, was 5.32×10^{10} cells/kg; the same pooled standard deviation, with 5 animals eliminated, was 1.86×10^{10} cells/kg. The data on all bone marrow studies in sheep, together with the procedures for analysis and calculation of data, were carefully reviewed just before preparing this report.

The results of the studies on the remaining 91 sheep are shown in Table 22. The first line of the table shows the mean total bone marrow cell count in unirradiated sheep. The values represent the pooled results of three separate studies, done at intervals of one year each. The marrow cell count was 2.74×10^{10} cells/kg, with a standard deviation of 1.83×10^{10} cells/kg. The second line shows the results of an early study after 150 R of acute irradiation; it is the only study in which sheep were irradiated for the sake of marrow studies alone. At 14 days after irradiation, the marrow cell count was only slightly more than half that of controls. This is about the same proportion as that of circulating granulocytes in sheep two weeks after acute irradiation in the midlethal range (see Chapter III).

The next four lines show the total marrow cell count in sheep during a typical chronic irradiation experiment. The protocol of irradiation for the experiment (Experiment 16) was given in Chapter II: 280 R at 3.4 R/hr, with two-week intervals between the beginning of each exposure. Marrow studies were done in various groups at five days after the completion of each dose increment. The first dose increment (280 R) resulted in an increase in the total marrow count, to almost twice the control value.

Table 22

TOTAL BONE MARROW CELL COUNT IN SHEEP FOLLOWING EXPOSURE TO ^{60}CO GAMMA RAYS

Original Experiment Number	EDR (R/hr)	Total Dose (R)	Postexposure Sample Time (days)	Number of Animals	Mean Total Marrow Cell Count*	SD*	Difference from Control	
							Cells*	Percent
17	(Control)	0	--	25	2.74	1.83	--	--
17	520	150	14	6	1.56	0.58	-1.18	-43%
16	3.4	280	5	8	5.14	3.17	+2.40	+88%
16	3.4	560	5	7	2.28	1.00	-0.46	-17%
16	3.4	840	5	8	1.56	1.60	-1.18	-43%
16	3.4	1,120	5	7	1.25	1.99	-1.49	-54%
8	3.7/533	950	240	7	1.60	0.83	-1.14	-42%
19	3.5/0.9	480	166	8	1.03	0.99	-1.71	-62%
20	3.5/0.45/10.3	1,355	8	8	2.60	2.92	-0.14	- 5%
21	10.3	1,071	9	7	0.76	0.68	-1.98	-72%

*Cells per kilogram x 10^{-10}.

After the second dose, the marrow cell count was only slightly less than the control value, and after the third and fourth doses the marrow count decreased further, to slightly less than half of the control marrow count. The rise in marrow count after the first exposure is comparable in magnitude to that seen in mice at 11 days after exposure (see Figure 12), and the total time in the sheep from the beginning of exposure to the determination of the marrow count was nine days. The measurements in the sheep after two, three, and four similar exposures indicate that such an upsurge in marrow count does not occur at a comparable time after each repeated increment. Note, however, that the mean bone marrow cell count after four dose increments was only slightly less than half the mean preirradiation count, even though the total dose amounted to an LD_{70}-LD_{80}.

The next three lines in Table 22 show the bone marrow cell count in sheep long after completion of an initial irradiation and during reirradiation. The first set of measurements was taken from Experiment 8. The animals were given an average of 810 R at 3.7 R/hr (20 R/day), and survivors were reirradiated with 140 R at 533 R/hr 90 days after completion of the first exposure. Bone marrow studies were made in survivors of the second dose eight months later. In the second set of measurements, taken from Experiment 19, the animals were given a mixed exposure of 100 R at 0.9 R/hr and 140 R at 3.5 R/hr each week for two weeks. Bone marrow studies were made in survivors at 166 days after completion of the irradiation. In the two groups of sheep, the total marrow cell counts were, respectively, 42 and 62% below the control levels, and this finding is consistent with the observation, documented in Chapter II, that sheep surviving irradiation retain a heightened sensitivity to irradiation for long periods of time. However, the third study in the group, taken toward the end of reirradiation of Experiment 20, seems to show an anomalous result. These animals had been exposed to 50 R/wk at 0.45 R/hr and 140 R/wk at 3.5 R/hr, to an overall average dose of 733 R. They were then further subjected to 120 R/wk at 10.3 R/hr, beginning 90 days after completion of the first irradiation series. At the time of beginning the second irradiation series, they were essentially equivalent to the animals of Experiment 19, and the bone marrow cell count would have been about 50%

of the control bone marrow cell count. Eight days after completion of five exposures in the second series, the bone marrow cell count was essentially equal to the control value, and the 50% loss before beginning the second series had apparently recovered.

The last line in Table 22 shows the bone marrow cell count in sheep of Experiment 21, which had been given 153 R per 2 weeks at 10.3 R/hr seven consecutive times. The bone marrow cell count was less than 30% of the control value. The total dose given represents about an LD_{80}-LD_{90}, and the bone marrow cell count shows extreme depression, more than that found in Experiment 16 after 1120 R. In comparing the results of the two experiments, it should be noted that the experimental evidence shows that the LD_{50} in Experiment 21 was lower than would be expected on the basis of ADR, indicating that recovery from the radiation injury was somewhat inhibited.

Significance of Bone Marrow Cell Counts for Mortality Effects in Mice and Sheep

Although the bone marrow is the tissue that figures most prominently in the determination of lethal effects of ionizing radiation in mammals, several lines of evidence suggest that the total amount of surviving marrow is not the sole factor in determining the lethal effects. These lines of evidence may be summarized briefly:

(1) The principal finding from the studies in mice was that at reduced values of EDR, the survival of cells was unaffected but the replacement of cells began at an effectively earlier time than after exposure at higher rates. The results imply that the time of commencement of marrow cell replacement may be an important factor in determining whether or not the animal survives the radiation dose.

(2) The mean total bone marrow cell count in unirradiated sheep was 2.74×10^{10} cells/kg. The mean total bone marrow cell count in unirradiated mice (average weight, 22.5 g) was 1.021×10^{9} cells. When the bone marrow cell count in mice was adjusted

to a per-kilogram basis, the count for mice was 4.55×10^{10} cells/kg. Thus, the sheep to mouse ratio of total marrow cells was about 0.6. In the same two species, however, the acute LD_{50} was about 265 R for sheep and about 900 R for mice. Thus, the ratio of LD_{50}s was about 0.3, about half the ratio for total bone marrow cells. Clearly, the difference in LD_{50} between the two species is not accounted for by differences in total bone marrow cell count.

(3) The series of studies of Experiment 16 show that the bone marrow of the sheep is able to recover rapidly from the first exposure to radiation but less rapidly from succeeding exposures. Still, five days after the fourth exposure, the total marrow count was 45% of the preirradiation value. This value was not much below that of sheep of Experiments 8 and 19--which were long-term survivors--but the sheep in Experiment 16 had received more than the LD_{50}.

(4) The studies with long-term survivors of irradiation (Experiments 8 and 19) show that total bone marrow cell count was depressed in these animals. These animals also had increased sensitivity to acute reirradiation. It would be natural to associate this increased sensitivity with the depressed state of the bone marrow cell count. However, when such animals were subjected to reirradiation on a chronic schedule (Experiment 20), the total bone marrow cell count apparently increased from its starting baseline, reaching the mean control level for unirradiated animals after five weekly doses of 120 R each. This total dose amounted to about an LD_{70} for the animals.

The results of Experiments 16, 19, and 20 suggest that ionizing radiation results in both destruction and proliferation of bone marrow cells. The proliferation is caused either by depletion of marrow cells, or by a direct effect of the radiation on the mechanisms controlling marrow cell proliferation rate, or both. The result is that, in Experiment 16, for instance, the total dose of 1120 R (four increments of 280 R) reduced

the marrow cell count by a factor of 10^6 (based on the results for mice), but the concurrent cell proliferation restored the marrow cell count to a level of about half the control value. The paradox is that three-fourths of the sheep in the group subsequently died, even though complete restoration of the total bone marrow cell count would have required only half a day of cell division time.

There are several possible explanations for this paradox. two of which are considered here. The first possible explanation involves the nature of mammalian cell renewal systems. These cell renewal systems usually consist of a number of morphologically distinct cell types, representing various stages of development and maturation, but there is also a functional division into what might be described as "germinal" cells and "production" cells. The germinal cells have the property that they can produce more germinal cells by mitotic division, and that they can differentiate into first-stage production cells. Production cells can also undergo mitotic division, but they cannot revert to germinal cells, and the mitotic division is coupled to a one-way maturation process that eventually leads to the terminal product-cells of the line, e.g., erythrocytes. In the bone marrow, the germinal cells are referred to as stem cells, which have not been morphologically identified, but are assumed to be, or to be related to, the colony-forming cells. The production cells of the marrow are the various myeloblasts, erythroblasts, megakaryocytes, and other cells that have been identified morphologically. Generally, the production cells of renewal systems grossly outnumber the germinal cells. The total number of colony-forming cells in the bone marrow of a mouse is probably between 10^6 and 10^7--between 0.1 and 1.0% of the total bone marrow cell count; hence changes in the total bone marrow cell count effectively represent only changes in the production cell count.

The question arises of whether there is a difference in the rates of replacement of production cells and germinal cells in the marrow. The present studies of replacement of total marrow cell count (i.e., production cells) in mice after acute irradiation at 500 R show that the total marrow cell count has a doubling time of 12.3 hr, after a time delay

of 37 hr. Earlier studies by Hanks and Ainsworth[14] on the replacement of colony-forming cells in the mouse after 450 R of X-rays showed that the colony-forming cells, as distinct from total marrow cells, had a doubling time of 31.9 hr, after a time delay of about 12 hr. Thus, where the total marrow cell count had been virtually restored in seven days after 500 R of gamma rays, the colony-forming cell count was still only 14% of its preirradiation value. These results and calculations indicate the possibility of a difference in the replacement rate of stem cells of marrow compared with production cells, in which the demand for production cells could lead to depletion and eventual exhaustion of the number of stem cells. In this case, the number of production cells in marrow a few days after a fatal radiation dose or dose increment might be only moderately depressed below control values, where the capacity for continued renewal of the cell population was gone.

The other possible explanation for the paradox of death of the animal at a time when bone marrow cell count is not severely depressed is that the irradiation seems to have some direct effect on the rate and degree of bone marrow proliferation during the postirradiation regeneration. The nature of the effect is not known, and hence most of the statements about it are necessarily speculative. It may be a direct effect on the surviving cells, or on the physiological mechanisms that control cell proliferation, or both. Two ways in which radiation appears to affect bone marrow cell proliferation are the following: It affects the time when cell replacement effectively begins, and it affects the "set point" which controls the total number of cells. The first effect is shown by the studies on mice, and the second effect is shown by the results of Experiments 8 and 19, where the total marrow cell count was below unirradiated control values for five to nine months after completion of the irradiation. Both of these effects are associated with lethal sensitivity of the animals to radiation. Other effects of radiation on the proliferation and regeneration of bone marrow, which are not revealed by the foregoing experiments, may also exist and may be associated with various aspects of lethal sensitivity.

V BIOLOGICAL AND MATHEMATICAL ANALYSIS OF LETHALITY IN LARGE ANIMALS EXPOSED TO IONIZING RADIATION

In this chapter the quantitative relationships among the parameters of EDR, ADR, and LD_{50} of large animals are considered. The data for constructing the relationships are drawn partly from the experimental data presented in Chapter II, and partly from other sources in the literature. Although the development of the relationships is mathematical in form, it must be understood that the basic approach is empirical. Too many exceptions to, and variances from, the relationships exist, and not enough is known about radiobiology of lethal effects to construct complete formal theories about the effect of radiation dose rate on LD_{50}. The overall aim is to attempt to reduce the widest possible range of available data to a useful set of generalizations. Comparable data for the mouse are also included since they provide information pertinent to interpretation of the large animal data.

Experimental and Analytical Procedures

Some reiteration of the experimental procedures described in Chapter II and some remarks on the analytical methods to be employed in this chapter appear appropriate.

The basic parameter of lethality or lethal dose is the LD_{50}. This is the dose, estimated by statistical analysis of mortality data from an experiment with a particular design, that will result in the death of 50% of a population of animals by the end of some arbitrarily set period after radiation exposure. In the following analysis, the LD_{50} at a particular dose rate is treated as an inherent property of the animal population, somewhat like a physical constant. This treatment is heuristically useful for developing generalizations about dose and dose rate, but the reader is reminded again that the LD_{50} is not a physical constant, but a number representing probability of death from radiation exposure.

The LD_{50} is actually defined in terms of an exact experimental procedure. Several groups of animals, matched as far as possible in distribution of biological variables, are exposed at a particular dose rate to different radiation doses in the lethal range. The data consist of the dose delivered to each group and the number of animals in each group that die during the time span of observation. The analysis consists of converting the percentage mortality in each group to probit of mortality and computing the regression of probit of mortality on logarithm of dose, using special statistical procedures.[2]

When the dose rate is low, so that the time over which the radiation exposure extends is long, the cost of the experiment can become excessive. In these circumstances, two shortcuts have sometimes been used:

Partial Exposure

A large group of animals are exposed to the radiation at the low dose rate (LDR), to a total dose that is less than the LD_{50}. The animals are then removed from the radiation field, and their LD_{50} is measured at a high dose rate (HDR), i.e., 500 to 600 R/hr, or more. The decrease in LD_{50} at the HDR from its normal value without prior LDR irradiation is the measure of the lethal effect of the LDR irradiation. If the accumulated injury is a linear function of the accumulated dose, then the LD_{50} at the LDR exposure is

$$LD_{50}\ (\text{LDR}) = \text{Total dose (LDR)}\ X\left[\frac{LD_{50}\ (\text{HDR})}{LD_{50}\ (\text{HDR}) - LD_{50}\ (\text{L/HDR})}\right] \qquad (4)$$

where LD_{50} (HDR) is the LD_{50} obtained for irradiation exclusively at the HDR, and LD_{50} (L/HDR) is the LD_{50} obtained for irradiation at the HDR after previous exposure at the LDR.

The assumption that accumulated injury is a linear function of the accumulated dose is critical to the analysis. Page, Ainsworth, and Leong[10] performed a series of partial exposures in sheep at 3.6 R/hr, followed by measurement of the LD_{50} at 660 R/hr in the formally correct experimental design. We have calculated the results of the partial

exposure series in terms of the percentage of the LD_{50} at HDR represented by each exposure at 3.6 R/hr, and the results are shown in Figure 13. The fitted line in the figure represents the linear regression of percentage LD_{50} on dose at 3.6 R/hr. The points show a considerable amount of scatter from the line, but the fitted line shows essentially zero injury at zero R, and predicts 100% LD_{50} at 488 R. The value of the LD_{50}, measured by the formally correct experimental design, was 495 R. Thus, at least for this experimental series, the assumption of linear accumulation of injury is confirmed, and the LD_{50} can be estimated from a single dose using Eq. (4).

It should be noted, however, that the particular lethality observed may have considerable effect on the estimation of LD_{50} from the formula given above. The estimations of LD_{50} from the individual points in Figure 13 range from 348 to 729 R. Such results show that the precision of estimation of the LD_{50} by this method is rather poor. For present purposes, only one experiment where the LD_{50} was estimated this way (Experiment 12) is cited in the analysis that follows.

Irradiation to Death

A large number of experiments have been performed in which groups of animals have been given continuous or repeated daily exposure to radiation at constant average rates until all of the animals were dead. The experimental data are the mean survival times of the animals of each group and the daily exposure rates of the groups. The original purpose of such experiments was to determine a rate of irradiation throughout lifetime that had no effect on the natural lifespan of the animals, and hence was "permissible" or "tolerable." From the viewpoint of this objective, such an experimental design is correct.

In a number of series of experiments, however, the ADR has ranged from very low values to quite high values, at which animals survived only a few days or weeks. We have used data from some experiments of this type, and have attempted to calculate LD_{50} values from the mean survival times to supplement our own data. The principle of the calculation is

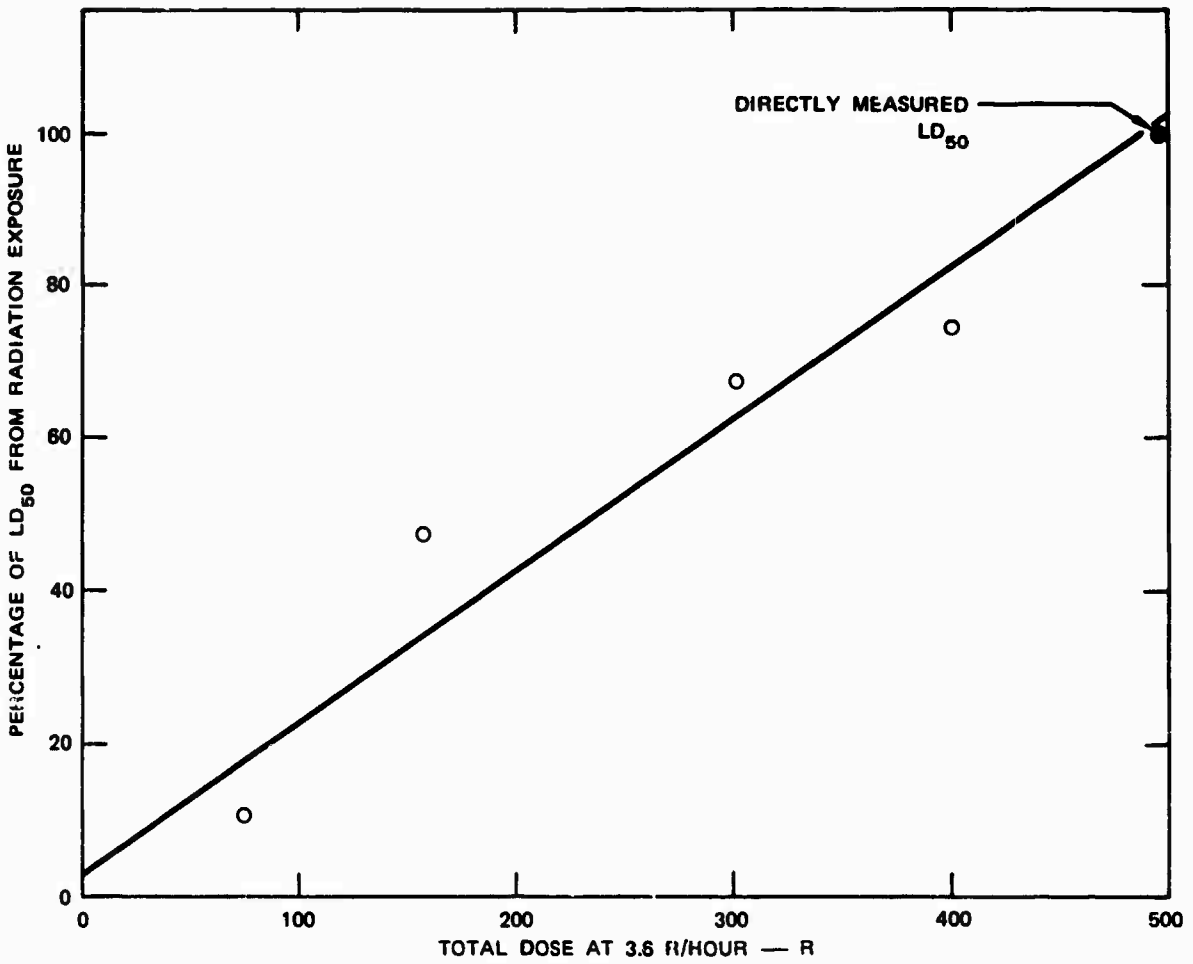

FIGURE 13 ACCUMULATED FRACTION OF THE LD_{50} FOR IRRADIATION TO VARIOUS DOSES OF ^{60}Co GAMMA RAYS AT 3.6 R/h

as follows: Assume that at some time in the course of exposure each animal attains a lethal radiation dose, and will die an average of X days later, whether the irradiation is continued or not. Then the mean survival time minus X is the mean time at which the animals in the group reach a lethal dose, and this time, multiplied by the daily exposure rate, is the mean lethal dose, which can be considered approximately equivalent to the LD_{50}. An alternate method of calculation, where the data are available, is to compute the cumulative percentage mortality with each new death, convert the percentage mortality values to probits, plot the probit of mortality against logarithm of dose, and interpolate the LD_{50} from this plot. This method is, in principle, the same as the ordinary computation of LD_{50} by the conventional experimental design.

The difficulty in the interpretation of experiments of this type is the determination of X, the mean time lapse from attainment of a lethal dose to the occurrence of death. Mean survival times after single acute exposures to radiation are about 25 days for the sheep and 16 to 18 days for the dog, rat, and pig. In one of the experiments cited in Chapter II (Experiment 4, Table 12), it was concluded that for sheep exposed continuously at 3.5 R/hr (87.4 R/day), probable mean survival time after attainment of a lethal dose was 19.0 days. In another study, by Still et al.,[5] it can be inferred in a similar way that for sheep exposed continuously at 1.96 R/hr (46.6 R/day), the probable survival time after attainment of a lethal dose was 29.2 days. These values are, respectively, 78 and 119% of the mean survival time after single doses of acutely lethal radiation at high dose rate in the sheep.

No similar data are available for other animal species. In interpreting the data on survival time during continuous irradiation in other species, we have used the data on the sheep as a guideline and have attempted to estimate a reasonable mean survival time after the lethal dose was reached. The procedure is admittedly crude, but reasonably satisfactory results can be obtained, and a number of values for mean lethal dose under chronic irradiation are thereby made available.

Effect of EDR on LD_{50}

In Chapter II it was stated that, for the sheep, it is conceptually useful to distinguish between those data where the significant parameter of radiation intensity is the intensity during actual exposure--i.e., the EDR expressed in R/hr--and those data where the significant parameter is the radiation intensity averaged over the entire exposure schedule--i.e., the ADR expressed in R/day. Obviously, these are identical for single continuous exposures but not for intermittent exposures. In this chapter the analytical consequences of this distinction between EDR-related and ADR-related data and its application to several species are elucidated.

Lethality as a Function of EDR

The data in this section are those obtained with EDRs ranging from tens to thousands of R/hr, and where a single continuous exposure was used.

Sheep

In 1970, Jones[15] summarized the data on LD_{50} values for sheep published in several places from different laboratories. The data are shown in Table 23. When the LD_{50} values were examined in relation to the EDR, it was found that there was a significant linear dependence of LD_{50} on EDR between 30 R/hr and 660 R/hr (Figure 14). The line in the figure, representing the linear regression of LD_{50} on EDR, corresponds to the equation

$$LD_{50} = 356 - 0.156\ EDR \qquad (5)$$

where the LD_{50} is in R, and the EDR is in R/hr.

Values of LD_{50} for 4 R/hr and below were considerably in excess of those predicted by Eq. (5) and were no longer a linear function of dose rate. This result implies that, in the sheep, another, or an additional, mechanism is operating at these low intensities to reduce the effectiveness of the radiation. This mechanism is conventionally considered to be associated with repair of injury during the radiation exposure. The

Table 23

RELATIONSHIP BETWEEN LD_{50} AND EDR IN SHEEP EXPOSED TO ^{60}CO GAMMA OR 1 MVP X-IRRADIATION

EDR (R/hr)	Type of Radiation	LD_{50} (R)	Reference
660	^{60}Co	237	Hanks[16]
578	^{60}Co	262	Table 1
573	^{60}Co	259	Table 1
450	1 Mvp	252	Hanks[16]
450	1 Mvp	314	Taylor[17]
450	1 Mvp	320	Still[4]
426	^{60}Co	298	Mobley[18]
261	^{60}Co	318	Page[10]
20	^{60}Co	338	Page[10]

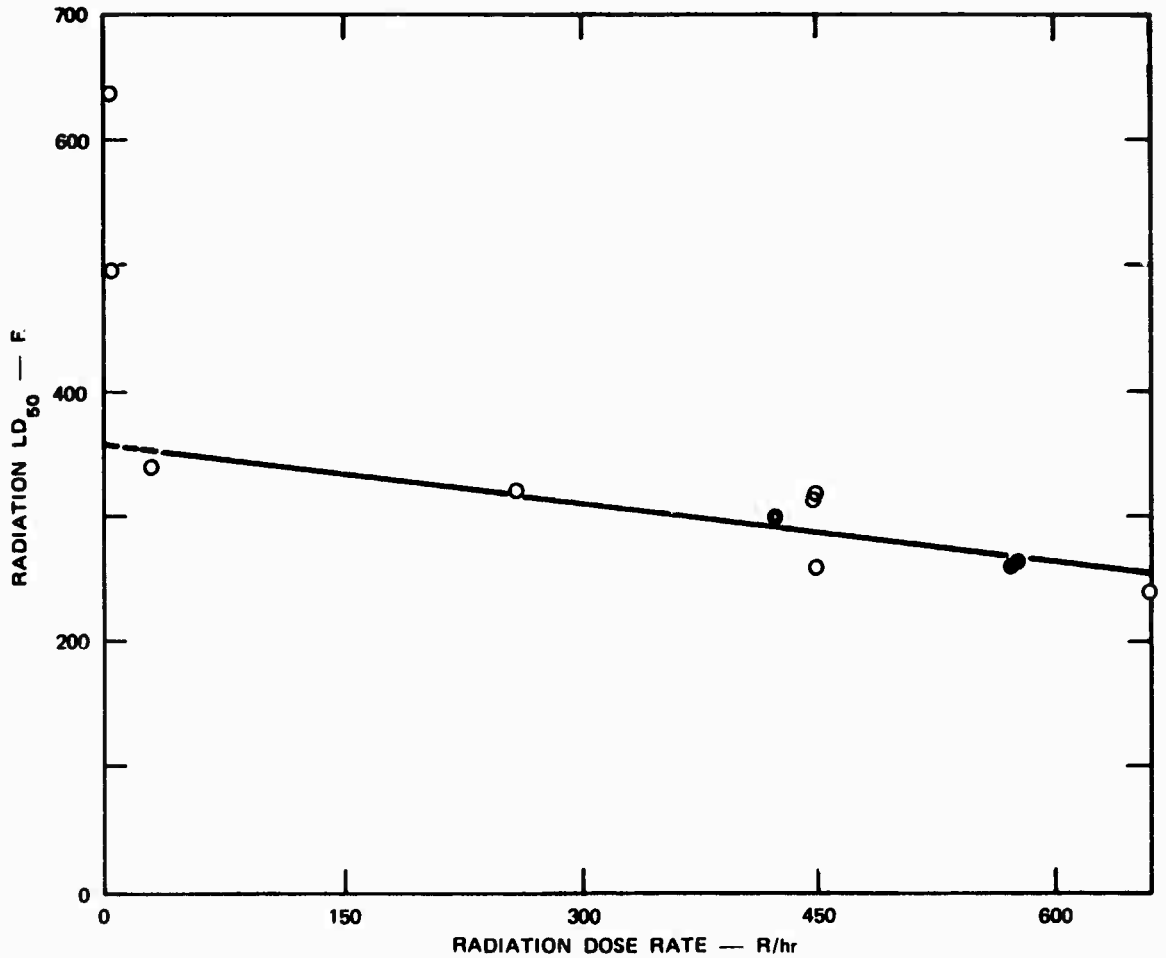

FIGURE 14 RELATIONSHIP OF LD_{50} TO RADIATION DOSE RATE IN THE SHEEP

lowest dose rate fitted to Eq. (5) was 30 R/hr; the LD_{50} was 330 R, and it required 11 hr to deliver. Thus, for the sheep, the additional repair mechanism is associated with lethal radiation times in excess of 11 hr.

Values of LD_{50} for dose rates greater than 660 R/hr were not available for analysis; however, it is clear that there must be some upper limit to the intensity range where Eq. (5) applies, or the LD_{50} will become zero or negative at a sufficiently high dose rate.

Swine

The available data on LD_{50} in relation to radiation dose rate for this species are summarized in Table 24. For consistency with the sheep studies, the data for pigs are restricted to those in which animals received radiation of 1 Mev energy or more, in an exposure configuration that was bilateral or multilateral. The data are drawn from a number of sources. Because of the diversity of sources of data, and because pigs vary greatly in size, a transformation of the data was made for the purposes of the present analysis. All the data on LD_{50} and dose rate, expressed as R in air, were converted to values of R at the midline of the animals, using the original authors' conversion ratios wherever possible. The mean conversion ratio for all the results given was 0.637. All the individually calculated values of midline dose rate and LD_{50} were then converted back to an average value in air by using the average conversion ratio of 0.637. The purpose in converting to midline values was to allow intercomparison of data from a variety of sources without the variability introduced by animal size, exact type of radiation, or conditions of exposure. The purpose in reconverting back to average values in air was to keep the data on the pig consistent with those on other species.

The reconverted data on LD_{50} and EDR are plotted in Figure 15. Dose rates go all the way up to 3000 R/hr. By inspection of the figure it can be seen that between EDRs of about 900 and 3000 R/hr,

Table 24

RELATIONSHIP BETWEEN LD_{50} AND EDR IN PIGS EXPOSED TO ^{60}Co GAMMA OR 1-2 MVP X-IRRADIATION

Type of Radiation	EDR		LD_{50}		Reconverted to Air		Reference
	Air (R/hr)	Midline (R/hr)	Air (R)	Midline (R)	EDR (R/hr)	LD_{50} (R)	
^{60}Co	3,000	1,920	375	240	3,015	377	Brown[19]
1 Mvp	1,800	882	510	250	1,385	392	Tullis[20]
^{60}Co	1,410	1,092	281	218	1,715	342	Chambers[21]
^{60}Co	1,410	1,040	310	228	1,630	358	Chambers[21]
2 Mvp	900	553	375	230	868	361	Tullis[20]
^{60}Co	651	399	381	233	626	365	Taylor[22]
^{60}Co	600	341	475	270	535	424	Brown[19]
1 Mvp	570	386	399	270	606	424	Nachtwey[23]
^{60}Co	275	168.5	507	311	264	488	Taylor[22]
^{60}Co	60	37.8	675	425	59.4	667	Brown[19]
^{60}Co	51	30.6	618	370	48.0	581	Rust[24]
^{60}Co	30	18.4	849	520	28.9	816	Taylor[22]

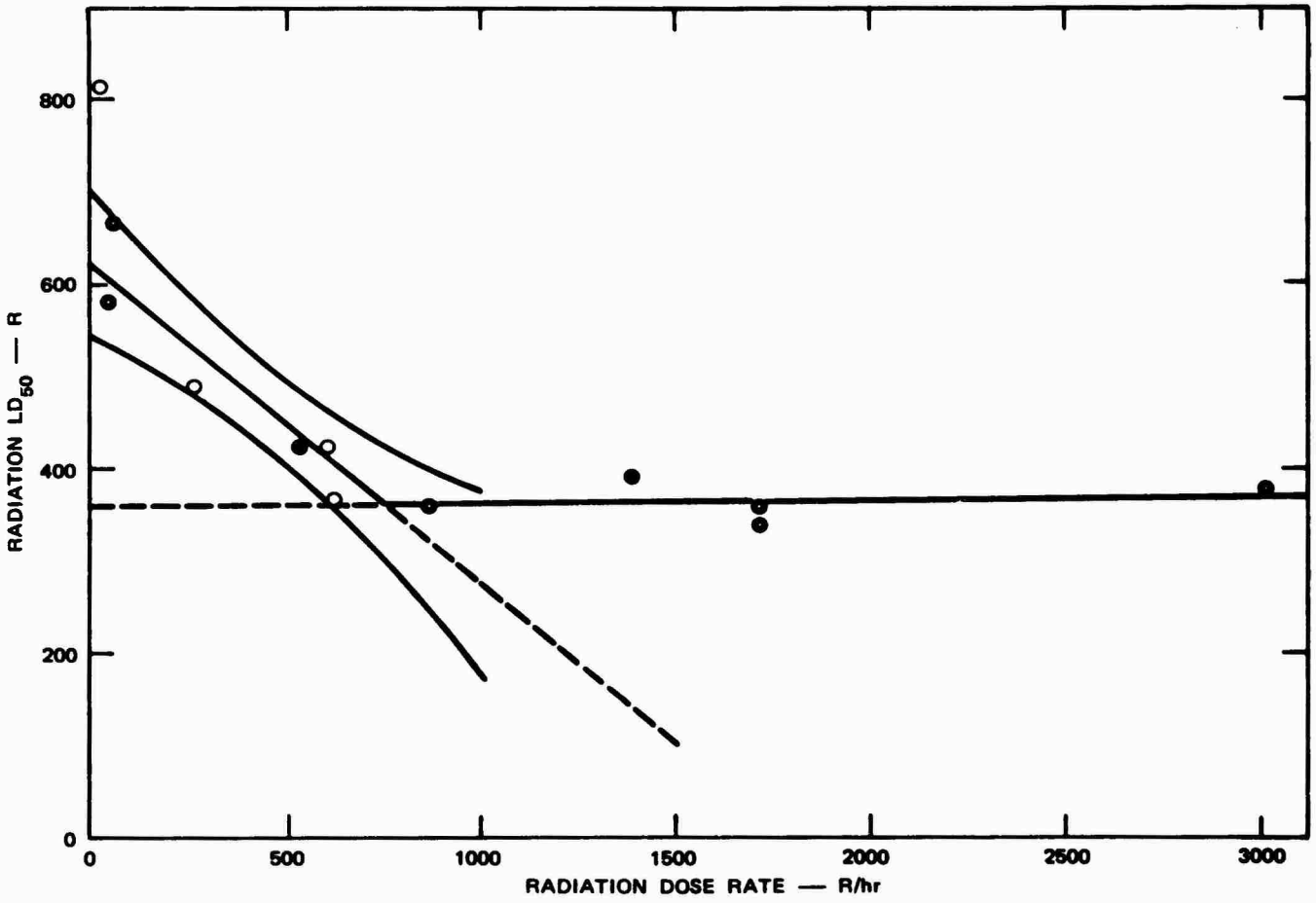

FIGURE 15 RELATIONSHIP OF LD_{50} TO RADIATION DOSE RATE IN THE PIG

there was little or no dependence of LD_{50} on dose rate. Calculation of the regression of LD_{50} on dose rate gives

$$LD_{50} = 359.2 - 0.0004 \text{ EDR} \tag{6a}$$

where the slope of the fitted line was 0.0004, with a 95% confidence interval of 0.043, confirming the independence of LD_{50} and EDR. Between 28.4 and 900 R/hr, there was a significant dependence of LD_{50} on EDR. The linear regression of LD_{50} on EDR was

$$LD_{50} = 683.7 - 0.442 \text{ EDR} \tag{6b}$$

with a 95% confidence interval of 0.235 for the slope. If the point at 28.5 R/hr was eliminated from the calculation, the regression of LD_{50} on EDR was

$$LD_{50} = 619.9 - 0.342 \text{ EDR} \tag{6c}$$

The slope of the new regression line was - 0.342, with a 95% confidence interval of 0.151. The expected value of LD_{50} at 28.9 R/hr from the new regression was 610 R, with a 95% confidence interval of 75 R. The measured value of LD_{50} at 28.9 R/hr was 816 R, greater than the expected value by almost three times the 95% confidence interval. For this reason, Eq. (6c) was selected as representing the dependence of LD_{50} on EDR, and the point at 28.9 R/hr was eliminated from the calculation of linear dose rate effects and placed with the data on protracted irradiation to be considered in a subsequent section.

The intersection point for Eqs. (6a) and (6c) was at 754 R/hr. The computed LD_{50} at this point was 362 R, and the time required to deliver the LD_{50} was 29 min. The result implies that, in the pig, there is an effect of dose rate on LD_{50} when the time required to deliver the LD_{50} is in excess of about 1/2 hour.

The lowest dose rate included in the regression was 48 R/hr. The measured LD_{50} at this dose rate was 581 R, and the LD_{50} expected from the regression of LD_{50} on dose rate was 603 R. The time required to deliver the LD_{50} was about 12.5 hr. The measured LD_{50} at 28.9 R/hr was 816 R, and the time required to deliver the LD_{50} was 28 hr. Thus, the additional repair mechanism operating at lower dose rates is characterized by a time for lethal irradiation of more than 12 but less than 28 hr.

Dogs

Compared with sheep and pigs, the available data on the relationship of LD_{50} to dose rate in dogs are very limited. The available data presented in Table 25 and Figure 16 are restricted to those in which the radiation was ^{60}Co gamma radiation or 1-2 Mvp X-rays, given bilaterally, quadrilaterally, or in a 4π exposure situation.

Only two points show any dependence of LD_{50} on EDR: at 360 and 540 R/hr. These points indicate a slope of 0.356 and an initial point of 511 R. Thus, relationship of LD_{50} to dose rate appears to be

$$LD_{50} = 511 - 0.356\ EDR \qquad (7a)$$

where LD_{50} is expressed in R, and the dose rate is in R/hr, both values in air. Between 900 and 3300 R/hr the regression of LD_{50} on EDR was

$$LD_{50} = 286.7 - 0.00009\ EDR \qquad (7b)$$

where the confidence interval of the slope, 0.00009, was 0.029, confirming the independence of EDR and LD_{50} in this region. The intersection of Eqs. (7a) and (7b) was at an EDR of 632 R/hr. The time required to give the LD_{50} (286.6 R) at this dose rate was 27 min. Thus it appears that dependence of LD_{50} on EDR occurs in the dog when the time to deliver the LD_{50} is 1/2 hour or longer. This result may be compared with that for the pig above.

Table 25

RELATIONSHIP BETWEEN LD_{50} AND EDR IN DOGS EXPOSED TO ^{60}CO GAMMA OR 1-2 MVP X-IRRADIATION

Type of Radiation	EDR in Air (R/hr)	LD_{50} Midline (R)	LD_{50} Air (R)	Reference
1 Mvp	3,300	250	285	Hansen[25]
2 Mvp	1,620	255	291	Bond[26]
1 Mvp	900	262	298	Gleiser[27]
1 Mvp	900	239	272	Bond[26]
1 Mvp	540	280	319	Ainsworth[28]
^{60}Co	360	336	383	Shively[29]

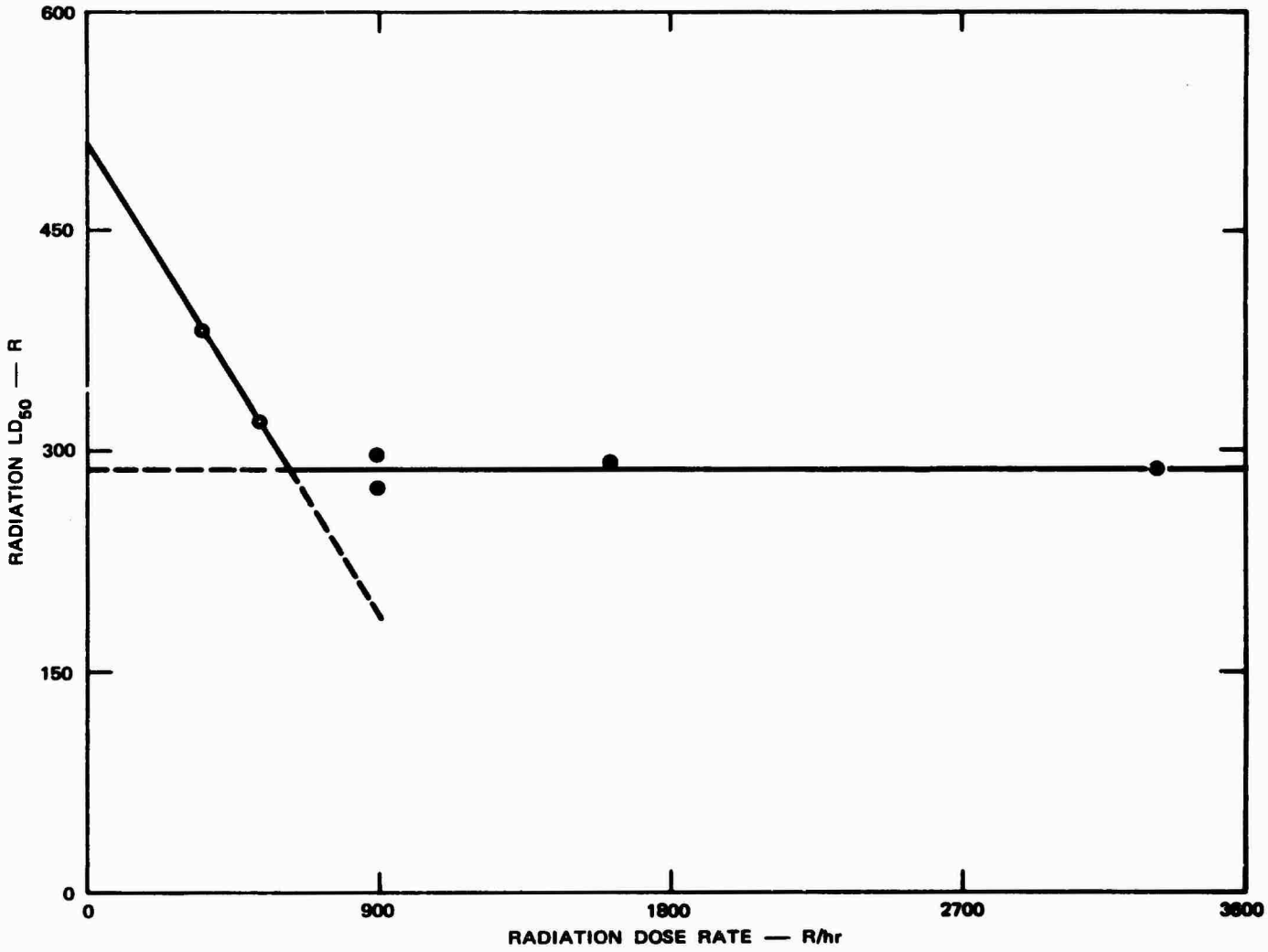

FIGURE 16 RELATIONSHIP OF LD_{50} TO RADIATION DOSE RATE IN THE DOG

Although some data on protracted irradiation of dogs are available, there are no data for exposure times that would indicate the upper time limit for the linear dependence of LD_{50} on dose rate, as are available for the pig.

Mice

Data from three sources showing the dependence of LD_{50} on dose rate in mice exposed to ^{60}Co irradiation are shown in Table 26. The plot of LD_{50} versus dose rate is shown in Figure 17. Between 1600 R/hr and 6000 R/hr, the regression of LD_{50} on dose rate is represented by

$$LD_{50} = 906.54 - 0.0013 \text{ EDR} \quad (8a)$$

Between 250 R/hr and 2000 R/hr, the regression of LD_{50} on dose rate is represented by

$$LD_{50} = 1164.44 - 0.155 \text{ EDR} \quad (8b)$$

The slope of Eq. (8a), 0.0013, has a 95% confidence interval of 0.037, indicating that in this region the LD_{50} is independent of dose rate. The slope of Eq. (8b), 0.156, is comparable in magnitude to that found for sheep above, and the 95% confidence interval is 0.017, indicating that there is significant dependence on LD_{50} on dose rate in this region. The point of intersection of Eqs. (8a) and (8b) is the EDR of 1667 R/hr, where the LD_{50} is 904 R, and the time to deliver the LD_{50} is 32.5 min. Thus, as in the pig and the dog, the LD_{50} in mice becomes dependent on EDR when the time to deliver the LD_{50} is more than about 1/2 hour.

By inspection of Figure 17 it can be seen that the points for dose rates below 250 R/hr do not follow the line represented by Eq. (8b). The linear regression of LD_{50} on dose rate in the region from zero to 250 R/hr is represented by

$$LD_{50} = 1437.38 - 0.851 \text{ EDR} \quad (8c)$$

Table 26

RELATIONSHIP BETWEEN LD_{50} AND EDR IN MICE EXPOSED TO ^{60}CO GAMMA RADIATION

EDR (R/hr)	LD_{50} (R)	Reference
5,870	899	Krebs[30]
3,091	905	Krebs[30]
1,758	873	Krebs[13]
1,604	932	Krebs[30]
1,012	1,012	Stearner[31]
821	1,029	Krebs[30]
697	1,046	Stearner[31]
543	1,086	Stearner[31]
405	1,095	Krebs[30]
369	1,106	Stearner[31]
282	1,127	Stearner[31]
202	1,209	Stearner[31]
202	1,287	Krebs[30]
189	1,359	Krebs[13]
156	1,249	Stearner[31]
84.6	1,354	Stearner[31]
58.6	1,407	Stearner[31]

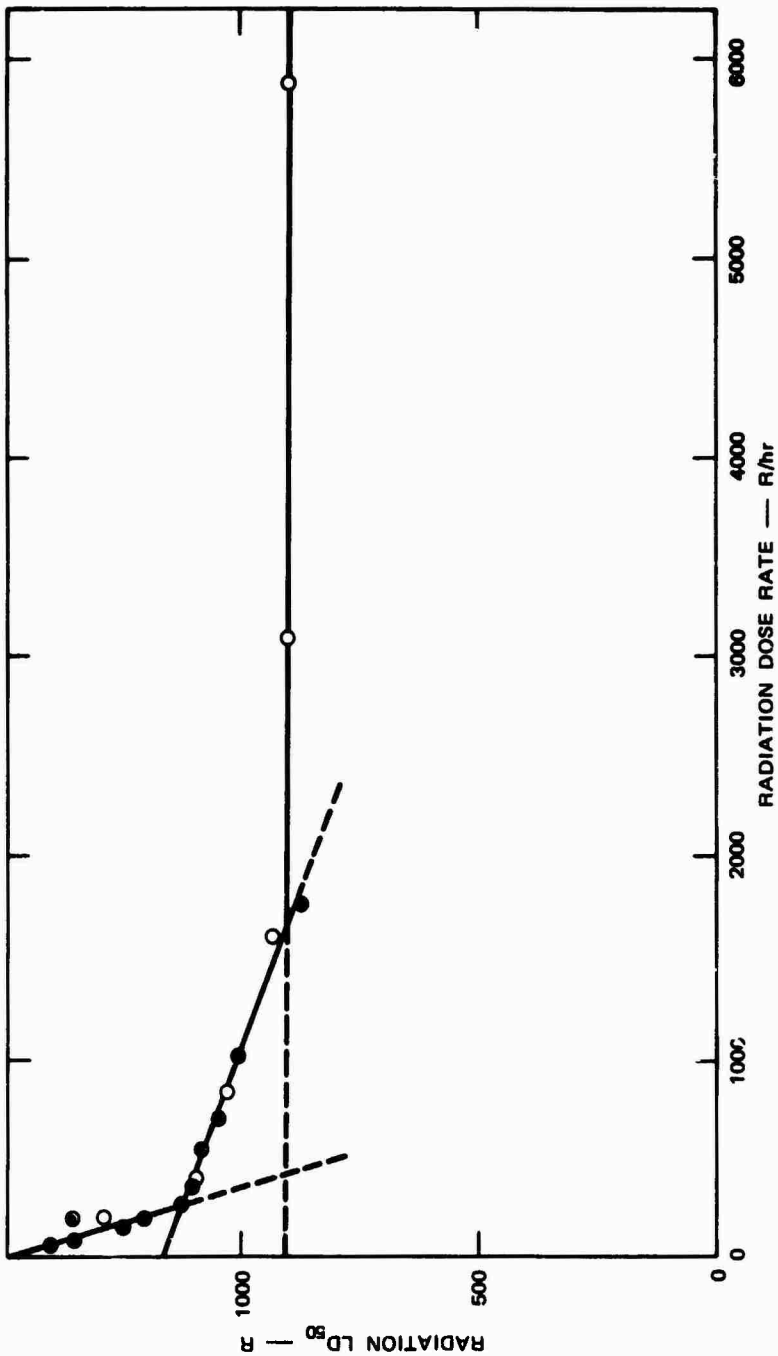

FIGURE 17 RELATIONSHIP OF LD_{50} TO RADIATION DOSE RATE IN THE MOUSE

but the slope of the line, 0.851, has a confidence interval of 1.183, indicating that the linear dependence is not a good fit. If the points at 202 R/hr and 189 R/hr with corresponding LD_{50}s of 1287 and 1359 R, respectively are eliminated from the data, the linear regression of LD_{50} on dose rate for the remaining points gives

$$LD_{50} = 1478.46 - 1.387\ EDR \qquad (8d)$$

and the 95% confidence interval of the slope is 0.545, indicating that the linear fit is reasonable. The intersection point between Eqs. (8b) and (8d) is the dose rate of 378 R/hr, with the corresponding LD_{50} of 1125 R and a time of delivery of 4 hr, 25 min.

Although the precise shape of the curve for exposure more than 3 to 4-1/2 hours is not clear, it appears that in this time range (3 to 30 hr), an additional mechanism for repair of radiation injury operates to increase the dependence of LD_{50} on EDR in the mouse.

Summary and Discussion

Considering the data of the previous section as a whole, the relationship of radiation LD_{50} to EDR appears to be divided into four regions:

(1) At high values of EDR, the LD_{50} is independent of the EDR.

(2) At medium to low values of EDR, the LD_{50} is a linear function of the EDR.

(3) Region 2 may sometimes be subdivided into two regions of linear dependence of LD_{50} on EDR, with separate slopes of the regression lines and separate times of appearance.

(4) At very low values of EDR, the linear dependence of LD_{50} on EDR breaks down, and an additional mechanism for repair of radiation injury arises, which further reduces the lethal effectiveness of the radiation.

Regions 1, 2, and 4 exist for all of the species examined, but region 3 was found only in the mouse. It is possible that region 3 depends on some special type of response of cells to irradiation and is restricted to species having this response.

The remarkable similarity in the relationship between EDR and LD_{50} for ionizing radiation among four animal species--representing three distinct orders of the Class Mammalia--suggests the existence of a fundamental process of adaptation to ionizing radiation that is common to the class as a whole. Of particular interest is the evidence that the dependence of LD_{50} on EDR is related to the duration of exposure of the animals. This result suggests that under the direct or indirect stimulus of irradiation, protective reactions are induced in the animal, and that the existence of EDR-dependent lethality response depends on the time required to mobilize these reactions.

The first EDR-dependent response occurs when the duration of exposure exceeds about 30 min. The evidence from studies of bone marrow cells of mice, presented in Chapter IV, indicates that this EDR-dependent effect is not related to either cell division during exposure or enhanced survival of cells. Instead, it seems to arise from some kind of mobilization of tissue-renewal processes that results in an earlier restoration of the marrow after completion of the exposure over a longer span of time. In Chapter IV, it is calculated that, on the basis of the earlier restoration of bone marrow, irradiation at 155 R/hr to a total dose of 500 R (3.23 hr of exposure time) resulted in a reduction in effectiveness of the radiation amounting to 94 R, or 19% of the dose given. On the basis of Eq. (8b), the expected EDR for reaching an LD_{50} in mice in the same amount of time (3.23 hr) would be 344 R/hr, the LD_{50} would be 1111 R, and the reduction in effectiveness would be (1111 - 906), or 205 R, about 18% of the total dose given. Thus, the estimates of reduced effectiveness of the radiation from LD_{50} measurements and from bone marrow cell counts are in close agreement.

Originally, it was expected that the studies of bone marrow colony-forming cells in mice would show that the EDR-dependent LD_{50} response resulted from repair of sublethal injury to cells by the process reported by Elkind and Sutton.[32] This mechanism, however, would have resulted in greater immediate survival of cells in animals exposed at lower values of EDR—a result that was not found. Hence, the initial linear dependence of LD_{50} on EDR (region 2) does not arise from "Elkind recovery." However, the intermediate region of dependence of LD_{50} on EDR in mice (region 3) might result from such a mechanism. In the analysis given in the preceding pages, this effect of EDR on LD_{50} begins when the duration of exposure exceeds about 4-1/2 hr. Studies by Till and McCulloch[33] indicate that recovery in bone marrow colony-forming cells by the Elkind process reaches its maximum value at around 5 to 7 hr after the initial irradiation, so the timing of EDR dependence in region 3 is reasonably consistent with such a mechanism.

At very low values of EDR, where the duration of exposure to lethal levels of radiation is of the order of one day or longer, an additional mechanism of recovery is activated (region 4). On the basis of the time spans involved, it may be reasonably supposed that the recovery in this region results from proliferation of the surviving cells during the exposure. Some aspects of the kinetics of proliferation in the bone marrow of sheep during irradiation are discussed in Chapter IV. The kinetics of repair from the viewpoint of accumulation of lethal injury are analyzed in the next section of this chapter.

Lethality as a Function of ADR

Sheep

It was noted in the previous section that, for sheep, the LD_{50} was related to radiation dose rate as shown in Eq. (5). This formula applied between 30 and 660 R/hr. However, Page, Ainsworth, and Leong[10] showed that for 3.6 and 2.0 R/hr, the LD_{50}s were 495 and 637 R, respectively, and data from Chapter II show that the LD_{50}s at 0.9 and 0.45 R/hr were 1251 and 1713 R, respectively. Clearly, these values would not be

expected from Eq. (5), and imply that at low values of EDR, the LD_{50} is further reduced below that predicted by the formula.

Two other observations are relevant to the analysis that follows. First, comparison of Experiments 8 and 11 in Chapter II (Table 4) shows that the same LD_{50} is obtained when radiation is given at 0.9 R/hr or 3.6 R/hr, provided that the same total dose is given each day. Second, experiments in which radiation is given at 3.6 R/hr in dose increments of 140 R/wk or 280 R/2 wk (ADR $\sim$ 22-24 R/day) give LD_{50} values that are somewhat, but not extremely, less than that obtained for 0.9 R/hr given continuously. These same LD_{50} values are considerably greater than that obtained for 3.6 R/hr given continuously (495 R). These results led to the conclusion that, at EDRs of 3.6 R/hr and below, the LD_{50} is independent of the EDR, and depends rather on the ADR.

The data used for the analysis of the relationship between ADR and LD_{50} are summarized in Table 27. The first two entries in the table are the LD_{50}s determined by Page, Ainsworth, and Leong[10] for 3.6 and 2.0 R/hr given continuously, and the remaining entries are from Chapter II of this report. For two of the experiments, multiple entries are made. In Experiment 19, it was noted previously that the LD_{50} was 680 R, but the mortality in one dose group was excessively large; without this group the LD_{50} would be 760 R. Both of these LD_{50} values are listed separately (lines 3 and 4). In Experiment 16, it was noted that the radiation doses with odd and even numbers of exposures appeared to give different values of the LD_{50}. The values for odd and even numbers of exposures, as well as the value for all exposures combined, are listed separately in Table 27 (lines 6, 8, and 10).

The analysis is contained in the sequence of columns of the table. Columns 1 and 2 show the ADR and EDR, in R/day and R/hr, respectively. The measured $LD_{50/60}$ in R at each ADR is shown in the third column. The fourth column, "Theoretical $LD_{50/60}$," is the $LD_{50/60}$ that would have been expected on the basis of Eq. (5). The fifth column, "Repair," is the difference between measured and theoretical $LD_{50/60}$ (columns 3 and 4).

Table 27

ANALYSIS OF REPAIR AND ACCUMULATION OF LETHAL INJURY IN SHEEP DURING EXPOSURE TO ^{60}CO GAMMA IRRADIATION AT LOW EDR

Dose Rate		$LD_{50/60}$		Repair	Exposure Time	Repair Rate	
(R/day)	(R/hr)	Measured (R)	Theoretical* (R)	(R)	(days)	(R/day)	(percent of ADR)
85.5	3.6	495	355.4	139.6	5.8	24.1	28.2
47.5	2.0	637	355.7	281.3	13.4	21.0	44.2
34.3	1.57	760.4	355.8	404.7	22.2	18.3	53.2
34.3	1.57	680.1	355.8	324.4	19.8	16.4	47.7
27.1	1.25	920.2	355.8	564.4	33.9	16.7	61.3
25.6	3.47	996.9	355.5	641.4	38.9	16.5	64.3
24.0	3.39	883.1	355.5	527.6	36.8	14.4	59.7
23.9	3.47	1,059	355.5	704.0	44.4	15.9	66.4
22.9	3.39	1,016	355.5	660.5	44.4	14.9	65.0
22.1	3.47	1,113	355.5	757.9	50.4	15.1	68.1
20.1	0.915	1,251	355.9	895.7	62.4	14.4	71.6
19.9	0.871	1,117	355.9	761.5	56.1	13.6	68.1
19.4	3.63	1,250	355.4	895.4	64.6	13.9	71.6
9.8	0.455	1,712	355.9	1,357	174.3	7.79	79.2

*Based on the formula: $LD_{50} = 356 - 0.156\ (EDR)$

The sixth column, "Exposure Time," is the total number of days required at the given ADR to deliver the measured $LD_{50/60}$. The seventh and eighth columns, "Repair Rate," are, respectively, the amount repaired per day of exposure and the percentage of the daily radiation exposure that is repaired. In summary, for the ADR of 85.5 R/day, the EDR was 3.6 R/hr, the measured $LD_{50/60}$ was 495 R, the theoretical $LD_{50/60}$ was 355.4 R (356 - 0.156 X 3.6), and the repair was 139.6 R, or 24.19 R/day, which was 28.2% of the rate of exposure.

Columns 2, 7, and 8 of Table 27 show that, as the ADR decreased, the absolute repair per day became a rapidly increasing proportion of the daily exposure rate. The overall effect suggests that: (1) the daily repair rate has some maximum value, which is approached asymptotically as the daily rate of exposure increases; and (2) the repair rate approaches the ADR as the ADR decreases, becoming equal to it at zero ADR. This relationship can be approximated by assuming that the repair rate is an exponential function of the exposure rate:

$$R = R_o \ (1 - e^{-k \ ADR}) \qquad (9)$$

where R is the rate of repair per day, R_o is the maximum repair per day, k is a constant, and ADR is the rate of exposure per day.

The method of fitting the data of Table 27 to Eq. (9) is as follows: (1) a reasonably probable value of R_o is assumed (from the data of Table 27, it was assumed to be 25 R/day); (2) the measured rate of repair per day is subtracted from the assumed value of R_o; and (3) a calculation is made of the linear regression of $\log_e (R_o - R)$ on the ADR [this regression calculation customarily includes the value of zero R/day exposure, for which $(R_o - R) = R_o$]. The computed slope of the regression equation is the estimated value of k.

From the first fit of the data to Eq. (9), a revised estimate is obtained for R_o, as follows: From the regression equation, the expected values of $\log_e (R_o - R)$ are computed for each of the measured ADRs.

The values of [expected (R_o - R) + measured (R)] are new estimates of the value of R_o; if the mean value of the new estimate of R_o is different from the one used previously, a new cycle of calculation is performed, starting with the new estimate of R_o. With repeated recalculations, the estimated value of R_o converges on some final value, which becomes accepted for general use.

The complete set of values in Table 27 was fitted to Eq. (9) by the procedure described above. The resulting regression line was

$$\log_e (24.92 - R) = 3.220 - 0.0396 \text{ ADR} \qquad (10)$$

and in the exponential form

$$R = 24.92 \ (1 - e^{-0.0396 \text{ ADR}}) \qquad (11)$$

A plot of the resulting relationship between ADR and recovery rate is shown in Figure 18. The points in the figure are the experimental points listed in Table 27. The curved line is the fitted line corresponding to Eq. (11), and the horizontal line is the maximum repair rate asymptote of the fitted line. It can be seen that the fitted line is an excellent representation of the data points.

Backfitting of the fitted Eq. (11) was done to obtain the expected values of repair rate, $LD_{50/60}$, and time to $LD_{50/60}$ for various values of ADR. Eq. (11) predicts the rate of repair of radiation injury at a given rate of radiation exposure. To calculate a predicted $LD_{50/60}$ for a given rate of exposure, the calculated rate of repair is subtracted from the rate of exposure, giving the net rate of accumulation of injury. The theoretical $LD_{50/60}$ for the ADR is divided by the rate of accumulation of injury, giving the calculated number of days required to reach the $LD_{50/60}$. The calculated number of days required to reach the $LD_{50/60}$, multiplied by the ADR, gives the calculated $LD_{50/60}$.

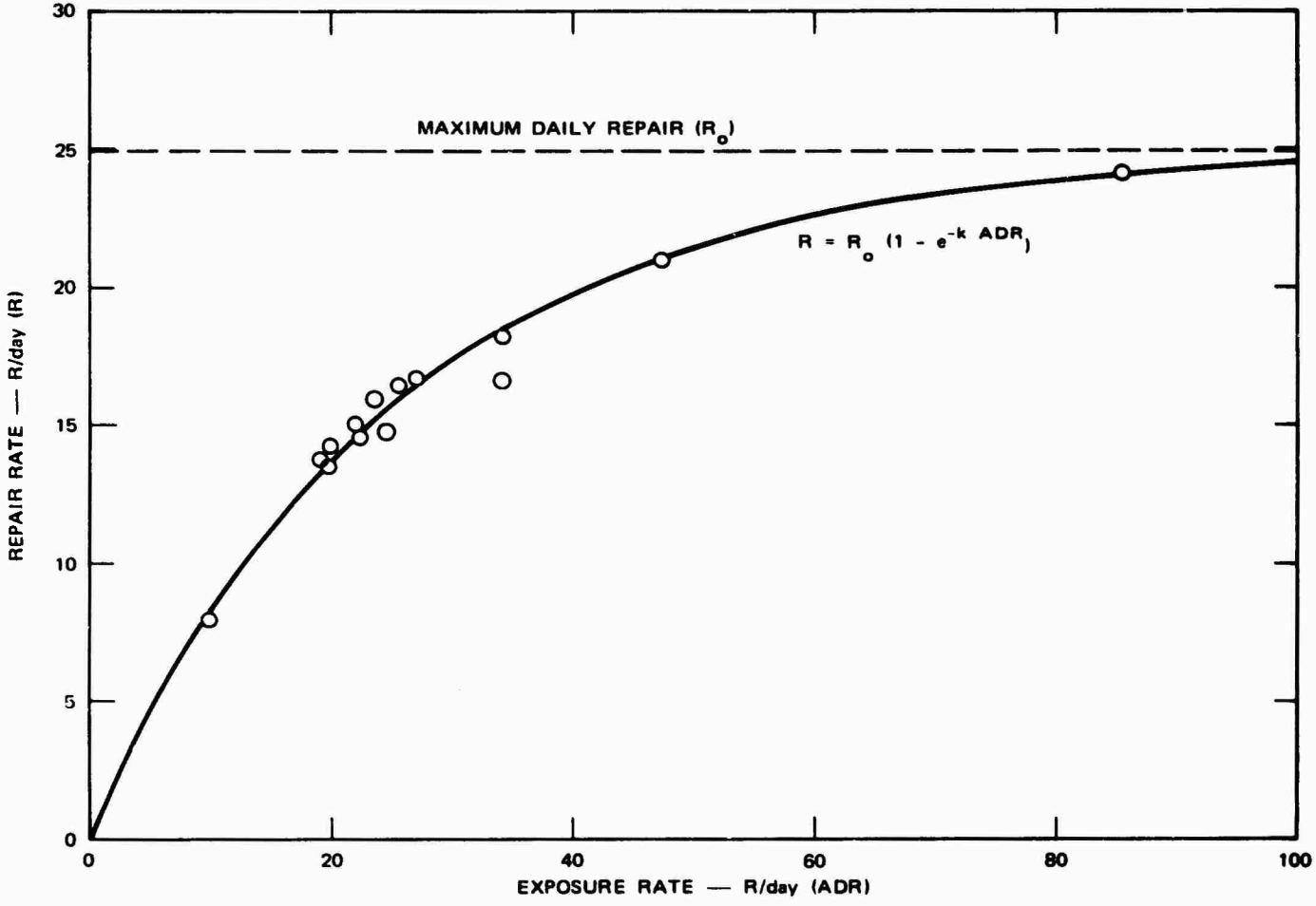

FIGURE 18 THE RATE OF REPAIR OF LETHAL GAMMA RADIATION INJURY IN SHEEP IN RELATION TO THE RATE OF RADIATION EXPOSURE (ADR)

The relationship calculated in this manner between $LD_{50/60}$ and ADR is shown in Figure 19. The points in the figure are the experimental points listed in Table 27, and the smooth curve is the result of the calculation described above. With the possible exception of the 10 R/day exposure, the agreement between calculated and measured $LD_{50/60}$ is excellent. At the 10 R/day exposure, the predicted rate of repair is in good agreement with the measured value, but at this exposure rate the repair is about 80% of the dose, and small uncertainties in the rate of exposure or the rate of repair can make large contributions to the rate of accumulation of lethal injury.

The foregoing analysis shows that the data on $LD_{50/60}$ in relation to ADR in the sheep for EDRs below 4 R/hr can be fairly represented by a model in which the rate of repair is assumed to be an exponential function of the exposure rate. Although the model gives excellent results, further consideration of the limits and applications is in order.

The model was developed for EDRs of 3.6 R/hr and below. In Chapter II, two sets of chronic exposure experiments are presented where the EDRs are greater than 3.6 R/hr: the exposures at 10.3 R/hr (Experiments 21, 23, and 20R) and the exposure at acute dose rates of 500 to 600 R/hr (Experiments 14 and 22). In both of these sets of experiments, there was clear evidence of reduced lethal effect of the radiation because of time protraction of exposure, and in both sets, the reduction in lethal effectiveness was less than that found for exposures at 3.6 R/hr or less.

Considering the acute experiments first, it can be noted that although the theoretical LD_{50} for the low dose rate studies is around 356 R (mean value in Table 27, 355.63), the equivalent values for Experiments 14 and 22 are 276 and 261 R, respectively. If the value of R_o, 24.92 R/day, represents maximum recovery for an LD_{50} base of 356 R, it would be reasonable to assume that its value might be scaled down in proportion to the LD_{50} base.

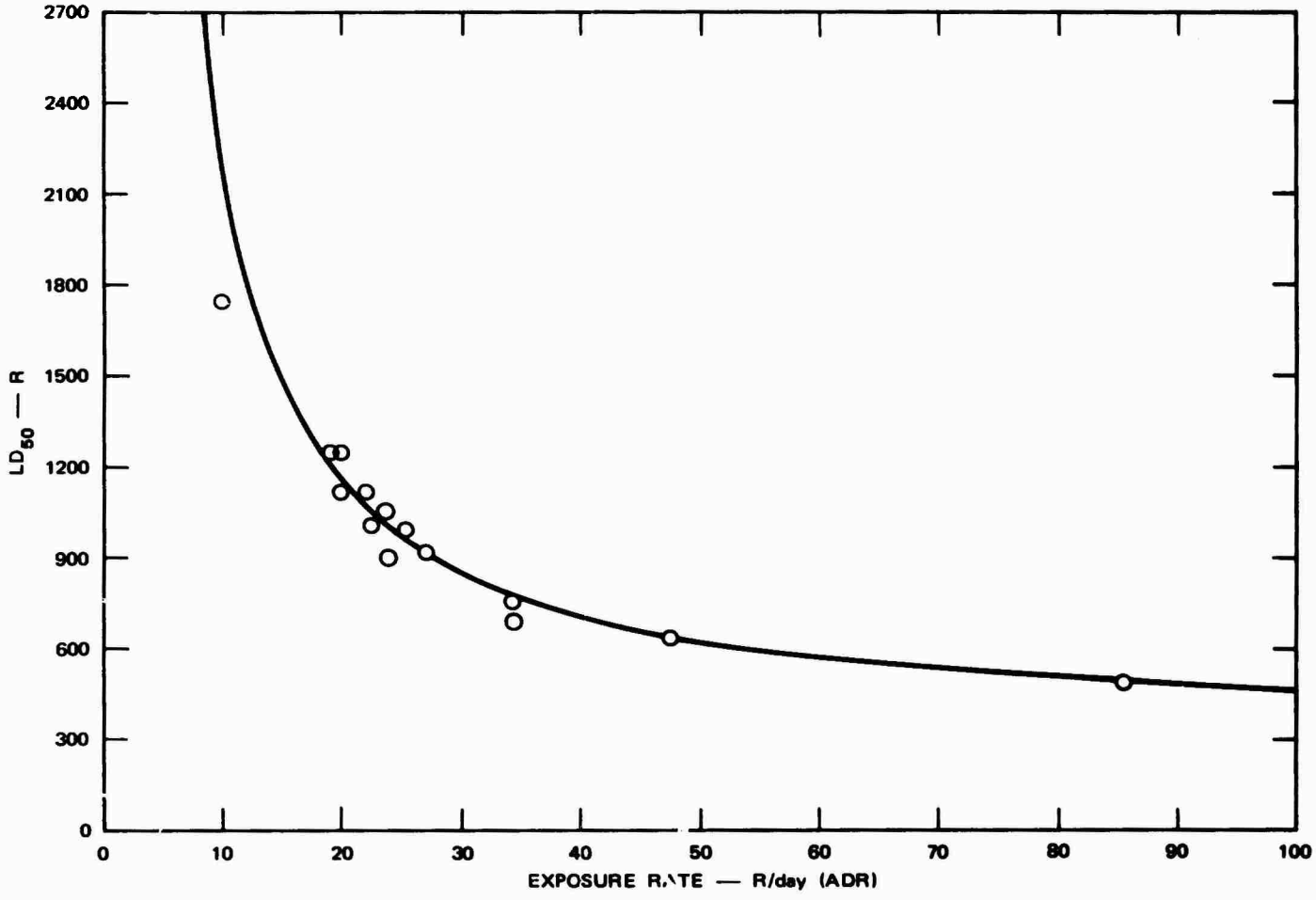

FIGURE 19 THE ACUTE LD_{50} IN SHEEP FROM GAMMA IRRADIATION IN RELATION TO THE RATE OF RADIATION EXPOSURE (ADR)

Table 28 shows the calculations and expectations resulting from such an assumption. The ADR for Experiment 14 was 100 R/day, and the ADR for Experiment 22 was 20.6 R/day, based on a median dose of 44 R given 15 times over a span of 32 days. The measured LD_{50}, divided by the ADR was the mean time to the LD_{50}. The theoretical LD_{50} was calculated by Eq. (5); the difference between theoretical and measured $LD_{50/60}$ was the recovery; and the recovery, divided by the mean time to the $LD_{50/60}$, was the mean recovery per day. These calculations are all identical to those used for the animals exposed at EDRs of less than 4 R/hr. Referring to Eq. (11), the value of R_o, 24.92, multiplied by the fraction (theoretical $LD_{50}/356$) gives the adjusted value of R_o corresponding to the value of EDR for each experiment. Using these adjusted values in Eq. (11), together with the appropriate values of ADR, gives the calculated daily recovery rate, which may be compared with the measured daily recovery rate derived above. For Experiment 22, the calculated and measured recovery rates are essentially identical. For Experiment 14, the measured recovery rate is somewhat larger than the calculated value. This experiment, however, involved not only a limited series of animal groups but also a very short time to reach the LD_{50}; hence, the discrepancy in calculated and measured values of recovery rate is insignificant. The calculated values of $LD_{50/60}$, obtained from the sum of the theoretical LD_{50} and the product of calculated recovery rate and measured time to $LD_{50/60}$, agree with the measured values of $LD_{50/60}$ to a good degree of tolerance. It is concluded from this analysis that the procedure of calculating daily recovery rate in protracted or repeated irradiation with a given value of ADR applies at any value of EDR, even very high ones, with the provision that the maximum daily recovery rate [R_o, Eq. (11)] is adjusted to the theoretical LD_{50} expected for the value of the EDR.

For the experiments at 10 R/hr, however, the deficit in recovery rate cannot be explained on such a basis. The status of recovery rate in these experiments is shown in Table 29. The expected recovery rate on the basis of the ADR is 10 R/day compared with the 6-7 R/day found.

Table 28

MEASURED AND CALCULATED PARAMETERS OF RADIATION INJURY AND RECOVERY IN SHEEP EXPOSED INCREMENTALLY TO ^{60}CO GAMMA RAYS AT EXPOSURE DOSES OF 513 and 607 R/HR

Parameter	Unit	Experiment 14	Experiment 22
Exposure dose rate (EDR)	R/h	513.0	607.0
Average daily rate (ADR)	R/day	100.0	20.6
Estimated $LD_{50/60}$	R	358.0	522.0
Mean time to $LD_{50/60}$	Days	3.6	25.3
Theoretical LD_{50}*	R	276.0	261.3
Recovery	R	82.0	260.7
Daily recovery rate	R/day	22.9	10.3
Revised maximum recovery rate, R_0	R/day	19.3	18.3
Expected recovery rate†	R/day	19.0	10.2
Expected LD_{50}	R	343.9	519.0

*Calculated from the formula: $LD_{50} = 356 - 0.156 \times EDR$

†Calculated from the formula: $R = R_0 \left(1 - e^{-0.0396 \times ADR}\right)$

Table 29

MEASURED AND CALCULATED PARAMETERS OF RADIATION INJURY AND RECOVERY IN SHEEP EXPOSED TO ^{60}CO GAMMA RAYS AT AN EDR OF 10.2 R/HR FOR 15 HOURS EVERY TWO WEEKS

Parameter	Unit	Experiment 21	Experiment 23
Average daily rate (ADR)	R/day	13.2	13.5
Estimated $LD_{50/60}$	R	811.7	638.0
Mean time to LD_{50}	Days	61.7	47.2
Theoretical LD_{50}*	R	354.4	354.4
Recovery	R	458.3	283.6
Daily recovery rate	R/day	7.4	6.0
Expected recovery rate†	R/day	10.1	10.3
Expected LD_{50}	R	1,531.5	1,502.1
Revised value of k‡	$(R/day)^{-1}$	0.0269	0.0204

*Calculated from the formula: $LD_{50} = 356 - 0.156 \times EDR$

†Calculated from the formula: $R = R_0 \left(1 - e^{-0.0396 \times ADR}\right)$

‡Calculated to make the following formula agree with observed values of daily recovery rate:

$$R = R_0 \left(1 - e^{-k \times ADR}\right)$$

From the expected recovery rate, the expected LD_{50} is about twice the value actually found. There was no significant difference in experimental procedure between these experiments and those at an EDR of 3.6 R/hr or less. The only difference in the experiments that can account for the difference in rate of recovery is that in the experiments at 10.2 R/hr, the total dose of 153 R per increment was delivered in 15 hr. The equivalent time to deliver the same size increment at 3.6 R/hr was 44 hr. Thus, it appears that recovery rate may depend on rate of accumulation of dose. On the basis of the experiments at 3.6 R/hr, the constants determined for Eq. (11) apply, when the actual dose in any single 24-hr period is not greater than 23-24% of the theoretical LD_{50}. Other restrictions for higher dose rates may also exist.

The last line in Table 29 shows a revised value of the constant k for Eq. (11). The value determined for the values of EDR less than 3.6 R/hr was 0.0396; the values in the table are those that would give the correct value of recovery for Experiments 21 and 23 when used in Eq. (11) with a value of R_o of 24.92 R/day. The two values of k found were 0.0269 and 0.0204. Using the mean of these values, a predicted LD_{50} was calculated for Experiment 20R, which involved irradiation at 10.2 R/hr for 11.5 hr (120 R) each week. The mean ADR was 21.22 R/day, the estimated recovery rate was 9.82 R/day, and the predicted LD_{50} was 659.4 R. The measured LD_{50} for this experiment was 553.2 R, and the difference, 106.2 R, can be attributed to the prior irradiation of the animals in Experiment 20R.

One other restriction on the formulation of injury and recovery in this section merits comment. In all of the reirradiated experiments, there was evidence of substantial injury remaining from irradiation at 0.45-3.6 R/hr for as long as 90 to 180 days after termination of the irradiation. Without considering the formulation itself, the apparent measured rates of recovery of injury shown in Table 27 should have resulted in complete dissipation of the injury at 90 days after termination of the irradiation. It would appear from the general evidence that the recovery process during irradiation is in part a function of the

fact of being irradiated, and in a sense it can be said that the radiation "drives" the recovery. The formulation of recovery rate as a function of exposure rate [Eqs. (9) and (11)] is in agreement with this concept. In the absence of irradiation, the recovery process is probably both slow and incomplete.

Pigs

Data on protracted irradiation of pigs are much scantier than those for sheep. The following sources were used for data points in the analysis of protracted irradiation:

(1) An experiment[22] to measure the LD_{50} at 30 R/hr. This point is the last item listed in Table 24 on the relationship of LD_{50} to EDR. It was noted in the analysis of EDR effects that the LD_{50} at this rate did not fit with the other points, and should be included in the analysis of protracted irradiation.

(2) An experiment[22] to measure the LD_{50} of pigs at 4 R/hr.

(3) An experiment[19] to measure the mean survival time of pigs exposed to 100 R/day at 28.2 R/hr. The mean survival time was 56 days, and 24 days was subtracted from the mean survival time to allow for mean survival time after a lethal dose. The 32 exposure days remaining gave an estimate of 3200 R for the LD_{50}.

(4) An experiment[34] to measure the mean survival time of pigs exposed to 50 R/day at 33 R/hr. The mean survival time was 204.5 days, and after subtracting 24 days for survival after the lethal dose, the time for the LD_{50} was 180.5 days, and the estimated LD_{50} was 9025 R.

The data on measured LD_{50} and rate of repair are summarized in Table 30. For the experiment at 693 R/day, the measured dose and exposure rate were converted to midline doses and then back to air doses, as was done with the acute LD_{50} measurements analyzed earlier. For the remaining experiments, the air doses as reported were used without conversion.

Table 30

ANALYSIS OF REPAIR AND ACCUMULATION OF LETHAL INJURY
IN PIGS DURING EXPOSURE TO ^{60}CO GAMMA IRRADIATION AT LOW EDR

Dose Rate		$LD_{50/30}$		Repair (R)	Exposure Time (days)	Repair Rate	
R/day	R/hr	Measured (R)	Theoretical* (R)			R/day	Percent Of ADR
693	28.9	816.3	610.0	206.3	1.2	172	25
100	28.2	3,200	610.3	2,590	32.0	80.9	81
94	4.0	3,444	618.5	2,826	36.6	77.2	82
50	33.0	9,025	608.6	8,416	180.5	46.6	93

*Based on the formula: $LD_{50} = 619.9 - 0.342$ EDR.

The rate of repair in relation to the ADR was analyzed for the data in Table 30 by the same procedure as was used above for sheep. The fitted equation for rate of repair in the pig was

$$R = 174.3\ (1 - e^{-0.00622\ ADR}) \qquad (12)$$

The comparison of the measured and calculated rates of repair and LD_{50} are summarized in Table 31. It can be seen that there was good agreement between measured and calculated values at all rates of exposure, with a factor of 14 between the highest and lowest exposure rates.

A comparison of Table 27 with Table 30 and of Eq. (11) with Eq. (12) shows that pigs differ strikingly from sheep in the capacity to repair radiation injury. The maximum repair rate for the pig, 174.3 R/day, is more than seven times that for sheep, 24.92 R/day. This difference leads to the conclusion that pigs are able to absorb fantastic amounts of low EDR irradiation without mortality. Note that the LD_{50} for pigs irradiated at high EDRs is less than twice that for comparably exposed sheep, and this difference may be partly accounted for by the marked difference in body size. Thus, the pig appears to have a specific resistance to protracted exposure to ionizing radiation.

Dogs

The data on protracted irradiation of dogs used in the present study are those from an ongoing project at the Argonne National Laboratory, conducted by Norris and Fritz.[35] In these experiments dogs were exposed to ^{60}Co gamma rays continuously over a 22-hr day to daily doses of 72, 50, 35, 17, 10, and 5 R/day. As of 1020 days of exposure, the mortality data in the 17, 10, and 5 R/day groups were incomplete. The mean survival times of these groups were estimated by computing the regression of the probit of cumulative mortality on the logarithm of the cumulative survival time for each day of death in each group. The estimate of extrapolated day for the probit of mortality equal to 5 is the mean survival time. The mean survival times of the groups in which the mortality was complete were estimated in the more conventional fashion.

Table 31

COMPARISON OF MEASURED AND CALCULATED VALUES FOR LD_{50} AND RATE OF REPAIR OF LETHAL INJURY IN PIGS RECEIVING PROTRACTED EXPOSURE TO ^{60}Co GAMMA IRRADIATION

	Rate of Repair		LD_{50}	
ADR (R/day)	Measured (R/day)	Calculated* (R/day)	Measured (R)	Calculated (R)
693	172	171.9	816.3	811.7
100	80.9	80.7	3,200	3,165
94	77.2	77.2	3,444	3,452
50	46.6	46.6	9,025	8,909

*Based on the formula: $R = 174.3\ (1 - e^{-0.00622\ ADR})$

The analysis of survival time, LD_{50}, and repair of radiation injury is shown in Table 32. The mean survival time after accumulation of a lethal dose in the form of protracted irradiation (the AST) was estimated by assuming that the survival time increased as a linear function of the ADR, as in

$$AST = 10.59 + 0.1674\ (100 - ADR) \tag{13}$$

This formula was developed by assuming that the effect of protracted radiation to death on the AST was similar in dogs and sheep, but that the AST was proportional to the mean survival time after acute irradiation--16.4 days for dogs[28] and 25.4 days for sheep (Table 1). The product of the ADR and the net time to the LD_{50} give the estimated LD_{50}, and the theoretical LD_{50} and repair rate were calculated as before. The fitted equation for repair of protracted radiation injury in the dog was

$$R = 25.18\ (1 - e^{-0.0474\ ADR}) \tag{14}$$

Comparing Eq. (14) with Eqs. (11) and (12) for the sheep and the pig, it can be seen that both the maximum repair per day (R_o) and the slope of the regression (k) in the dog are much closer to the values for the sheep than for the pig.

The comparison of the measured and calculated rates of repair per day and LD_{50} are shown in Table 33. It can be seen that the rates of repair and the LD_{50}s are reasonably predicted by Eq. (14) down to about 17 R/day. Below this value, systematic discrepancies appear, where the measured LD_{50}s are less than those predicted from Eq. (14). Part of this discrepancy may reflect the fact that at low values of ADR, the recovery rate is nearly equal to the ADR, and small uncertainties in the values of the constants in Eq. (14) may result in large errors in estimating the net rate of accumulation of lethal injury. Part of the discrepancy probably also reflects the fact that with prolonged irradiation, other causes of death may arise that are unrelated to the accumulation of lethal injury at the higher values of ADR. Norris and Fritz[35]

Table 32

SURVIVAL TIME, LD_{50}, AND REPAIR OF LETHAL RADIATION INJURY IN DOGS EXPOSED TO ^{60}CO GAMMA RADIATION

Dose Rate		Mean Survival Time		Net Time To LD_{50}	LD_{50}		Repair	Repair Rate
R/day	R/hr	Total (days)	Post-LD_{50} (days)	(days)	Measured	Theoretical*	(R)	(R/day)
72	3.13	26.0	15.3	10.7	770.4	509.9	260.5	24.3
50	2.27	38.1	19.0	19.1	955.0	510.2	444.8	23.3
35	1.59	55.1	21.5	33.6	1,176.0	510.4	665.6	19.8
17	0.775	186†	24	162	2,754	511	2,243	13.8
10	0.455	460†	26	434	4,340	511	3,829	8.82
5	0.228	1,743†	26	1,717	8,585	511	8,074	4.70

*Based on the formula: $LD_{50} = 511 - 0.356$ EDR.

†Estimated from incomplete data.

Table 33

COMPARISON OF MEASURED AND CALCULATED VALUES FOR LD_{50} AND RATE OF REPAIR OF LETHAL INJURY IN DOGS RECEIVING PROTRACTED EXPOSURE TO ^{60}Co GAMMA IRRADIATION

ADR (R/day)	Rate of Repair		LD_{50}	
	Measured (R/day)	Calculated* (R/day)	Measured (R)	Calculated (R)
72	24.3	24.4	770.4	770.5
50	23.3	22.8	955.0	938.8
35	19.8	20.4	1,176.0	1,222.5
17	13.8	13.9	2,754	2,831
10	8.82	9.51	4.340	10,329
5	4.70	5.31	8,585	--

*Based on the formula: $R = 25.18\ (1 - e^{-0.0474\ ADR})$

note, for instance, that at lower values of ADR, a myeloproliferative disease is associated with deaths occurring after about 400 days of irradiation.

Mice

A large amount of data are available on the survival of mice exposed to repeated or chronic X-rays or gamma radiation. For the present analysis to be effective, it is necessary to be somewhat selective. The data selected for this analysis are the survival times of female LAF_1 mice exposed daily to ^{60}Co gamma irradiation at ADRs ranging from 145 down to 56 R/day, as reported by Sacher and Grahn.[36] The original publication covers a much wider range of ADR values, but analysis of the entire range would be both difficult and, for the present purposes, unnecessary.

Two problems arise in connection with the analysis. The first problem concerns the length of time the mice survive after reaching a lethal dose at any given ADR. For analysis of the present data, we have used

$$AST = 21.79 - 0.093\ ADR \tag{15}$$

Eq. (15) is based on considerations of the survival time of mice after single high EDR doses and on the effect of irradiation to death on the survival time of sheep, which was discussed previously. The constants in Eq. (15), however, were derived by backfitting from expected values of recovery and mean lethal dose, and represent the best fit, on the assumption that the kinetics of repair of radiation injury in the mouse are similar to those of the sheep, the dog, and the pig.

The second problem in analysis is the appropriate equation for predicting the expected theoretical LD_{50} for a given value of EDR. Three equations showing dependence of LD_{50} on EDR in mice are given above. On the basis of a judgment of the maximum amount of repair to be expected per day, we have used Eq. (8d).

The data on the survival times of mice, and their analysis in terms of estimated LD_{50} and rate of recovery, are shown in Table 34. Using the methods of analysis of daily repair rate applied previously to the sheep, the dog, and the pig, the daily repair rate in the mouse can be fitted to

$$R = 51.66\ (1 - e^{-0.032\ ADR}) \qquad (16)$$

Eq. (16) was fitted for values of ADR between 145 R/day and 56 R/day, as shown in Table 34. The comparison between the values of LD_{50} and daily repair rate derived from the data and the values calculated from Eq. (16) are shown in Table 35. From 145 R/day down to 56 R/day, the measured and calculated values are in reasonable agreement. Beginning at 56 R/day, and proceeding to lower values of ADR, the calculated values of rate of repair become systematically larger than the measured values, and the calculated values of LD_{50} become grossly larger than the measured values. In the original calculations, all the data on daily repair rate and ADR from 145 R/day down to 24 R/day were fitted to a general repair formula similar to Eq. (16). This procedure, however, gave a repair formula that was a poor fit for all values of ADR. The present form of Eq. (16) is a reasonable fit to the data from 145 R/day down through 64 R/day, but not at 56 R/day and below. This condition can be compared with that in the dog, noted previously.

General Summary: Effect of Radiation Dose Rate on the Lethal Dose of Radiation in Several Animal Species

As radiation dose is given over a progressively longer period of time, there is a general decrease in the lethal effectiveness of the radiation, so that larger total doses must be given to cause death of animals. This time-dependent effect is usually attributed to the fact that the injury can be repaired during the time of exposure. However, the nature of the repair process is not well understood, and, in general, when repair of radiation injury is indicated in the quantitative sense, the word "repair" really means "net reduction of effectiveness."

Table 34

SURVIVAL TIME, LD_{50}, AND REPAIR OF LETHAL RADIATION INJURY IN MICE EXPOSED TO ^{60}CO GAMMA RADIATION

Dose Rate		Mean Survival Time		Net Time To LD_{50} (days)	LD_{50}		Repair (R)	Repair Rate (R/day)
R/day	R/hr	Total (days)	Post-LD_{50} (days)		Measured	Theoretical*		
145	11.6	23.8	8.3	15.5	2,250	1,462	788	50.8
125	10.0	30.6	10.2	20.4	2,550	1,465	1,085	53.2
97	7.8	41.2	12.8	28.4	2,760	1,468	1,292	45.5
85	6.8	50.0	13.9	36.1	3,070	1,469	1,601	44.5
74	5.9	65.3	14.9	50.4	3,722	1,470	2,252	44.6
64	5.1	85.7	15.8	69.9	4,460	1,471	2,989	42.8
56	4.5	103.7	16.6	87.1	4,880	1,472	3,408	39.2

*Based on the formula: $LD_{50} = LD_{50} = 1,478 - 1.387$ EDR.

Table 35

COMPARISON OF MEASURED AND CALCULATED VALUES FOR LD_{50} AND RATE OF REPAIR OF LETHAL INJURY IN MICE RECEIVING PROTRACTED EXPOSURE TO ^{60}Co GAMMA IRRADIATION

	Rate of Repair		LD_{50}	
ADR (R/day)	Measured (R/day)	Calculated* (R/day)	Measured (R)	Calculated (R)
145	50.8	51.0	2,250	2,255
125	53.2	50.4	2,550	2,453
97	45.5	48.6	2,760	2,942
85	44.5	47.2	3,070	3,303
74	44.6	45.2	3,722	3,777
64	42.8	42.8	4,460	4,441
56	39.2	40.2	4,880	5,217
49	36.2	37.4	5,700	6,222
43	32.2	34.4	5,900	7,370
32	24.8	27.1	6,550	9,633
24	18.2	19.9	6,100	8,640

*Based on the formula: $R = 51.66\ (1 - e^{-0.032\ ADR})$

In expressing the dependence of lethal dose on time of delivery of the radiation, two expressions of dose rate have been used: EDR, which is the ratio of dose to time during the actual exposure to the radiation; and ADR, which is the total dose divided by the number of days over which it was delivered, irrespective of the actual dose or EDR per day. It has been shown that both EDR and ADR affect the lethal dose of radiation by processes that appear to be kinetically different.

As the EDR decreases from high values, the lethal dose is at first independent of EDR, and at lower values, it becomes an approximately linear function of EDR. The point at which the lethal dose becomes dependent on EDR is the value of EDR for which delivery of the lethal dose requires longer than about 30 min. This time mark is consistent among the dog, the pig, and the mouse; similar data were not available for the sheep. In lethal exposures requiring more than 30 min, the LD_{50} is related to the EDR by

$$LD_{50} = A - m\ EDR \tag{17}$$

The values of A and m vary with the species, but the extrapolated value of LD_{50} when the EDR equals zero ranges from 130 to 180% of the EDR-independent LD_{50}.

These values are far less than observed values of lethal dose when the radiation exposure is distributed over periods of several days to several months. In various species, lethal radiation doses have ranged from 7 to 25 times the EDR-independent values for moderate values of ADR. It is concluded that when the duration of irradiation exceeds some time period of the order of one day, other repair mechanisms are activated that reduce the effective daily radiation dose that is accumulated.

As the EDR becomes low, lethality becomes less dependent on EDR, and the ADR becomes the dominant parameter. The repair rate for prolonged exposure is related to the ADR as in

$$R = R_o\ (\ 1 - e^{-k\ ADR}) \tag{9}$$

where R is the repair rate in R/day, R_o is the maximum repair rate, ADR is the mean daily exposure rate, and k is a constant. In comparing among the species, R_o is about 25 R/day for the sheep and the dog, 50 R/day for the mouse, and 174 R/day for the pig. The value of 174 R/day for the pig represents an exceptionally high repair rate, and the pig has a very large resistance to irradiation protracted over a long time period, even though its resistance to single acute radiation doses is about the same as that of other large animal species. The R_o value of 50 R/day for the mouse is higher than that of the dog and the sheep, but, relative to the EDR-independent LD_{50}, the mouse is not exceptionally resistant to protracted irradiation.

Using Eq. (9) and the appropriate values of R_o and k, the recovery rate can be calculated for a specified ADR. The difference, (ADR - R), is the rate of accumulation of lethal injury per day. The expected single-dose LD_{50} [determined from Eq. (17)] divided by the rate of injury accumulation per day gives the expected time to accumulate a lethal dose. This time, when multiplied by the ADR, gives the calculated LD_{50} for the given ADR. Also, the time to accumulate a lethal dose, plus the time of survival after the lethal dose ($\sim$ 8-25 days), gives the calculated survival time of the animals.

Measured and calculated values of daily recovery rate, LD_{50}, and survival time are in good agreement for a fair range of values of ADR in the sheep, the pig, the dog, and the mouse. As values of ADR reach lower levels, the measured survival times in the dog and the mouse begin to become progressively less than predicted values. The reason for this digression is not clear. It may reflect uncertainties in the values of the constants R_o and k. It is probable, also, that at sufficiently low values of ADR and sufficiently protracted irradiation times, other specific causes of death or general reduction in life span should occur, and these causes of death may be governed by other rate mechanisms than those described in Eq. (9). In the dog and the mouse, the divergence between measured and predicted values of mean survival time occur when the times of radiation exposure exceed 400 and 100 days, respectively. These times represent between 10 and 16% of the average natural life spans of the species.

Applications to Man

The analysis presented in this chapter shows that the relationship between lethal dose of radiation and rate of exposure is essentially the same for all of the species studied. Differences among the species are expressed primarily by differences in the values of the constants of Eqs. (9) (repair vs ADR) and (17) (LD_{50} vs EDR). We would expect that in man, the relationships between lethal radiation dose and rate of exposure would be similar to that of the sheep, the pig, the dog, and the mouse. The problem remains to estimate the values of the constants in Eqs. (9) and (17) for man.

The most comprehensive study of radiation LD_{50} in man is that of Lushbaugh, Comas, and Hofstra,[37] which is an assembly of observations of humans accidentally exposed to radiation and of terminal human cancer patients treated (whole-body) with gamma rays. For 83 of the 100 cases, the EDR was between 45 and 96 R/hr. The LD_{50} for the entire group, computed from the probit-log dose regression, was 480 R of exposure or 316 rad of midline ("epigastric") tissue dose, and the midline/air ratio was 66 rads per 100 R of exposure. At the range of values of EDR for the major part of the group, the LD_{50} required between 5 and 11 hours of radiation exposure; hence, on the basis of the previous studies in the sheep and the pig, the LD_{50} found for man[37] would be in the EDR range where the LD_{50} was related to EDR by Eq. (17) and was not significantly dependent on ADR.

These data may be used in two ways to estimate the constants in Eq. (17) for man. In the first approach, it may be noted that for the same values of EDR, the LD_{50} would have been 340 to 350 R for the sheep, 580 to 605 R for the pig, and 475 to 495 R for the dog. On the basis of the closest match in LD_{50}, then, we might reasonably assume that the values of constants in Eq. (17) for man are similar to those for the dog: A (y-intercept) = 500 to 520 R, and m (slope) = 0.35 to 0.36 hr^{-1}.

The previous studies in dogs, pigs, and mice showed that the LD_{50} becomes independent of EDR when the exposure time for the LD_{50} is equal to or less than 30 min, i.e., when the numeric value of the EDR becomes

twice the numeric value of the LD_{50}, or more. Assuming that this relationship holds for man as well, and assuming the values of constants for man deduced above, we would conclude that in man, the LD_{50} becomes EDR-independent at between 590 and 610 R/hr, and the LD_{50} at this point and beyond is between 295 and 305 R (EDR-independent LD_{50}).

On the basis of 66 rads midline ("epigastric") dose per 100 R, the EDR-independent LD_{50} in man would be between 195 and 201 rads, midline. The comparable values in the dog and the pig were 240 and 220 rads, respectively. Hence, the value of the EDR-independent LD_{50} estimated above for man may be slightly low.

The second approach to estimating the constants for Eq. (17) in man entails assuming that the EDR-independent LD_{50} in man is around 230 rads, the average for the pig and the dog given above. The EDR-independent LD_{50}, in terms of exposure, would then be about 350 R, and the minimum EDR at which the LD_{50} became EDR-independent would be 700 R/hr. With this point, 350 R and 700 R/hr, and the other point given by Lushbaugh et al.,[37] 480 R and 45 to 96 R/hr, the constants for man in Eq. (17) can be estimated in a way that is independent of the first approach. The values for the second approach are: A (y-intercept) = 490 to 500 R; m (slope) = 0.20 to 0.22 hr^{-1}. Thus, the value of A is about the same for each of the two approaches to estimating the constants, but the value of m is lower in the second approach than in the first. The lower value of m indicates a less rapid decrease of LD_{50} with increasing EDR.

No similar data are available to provide guidelines for estimation of the constants of Eq. (9) (ADR versus recovery) for man. However, among the animal species studied, only the pig showed any exceptional resistance to chronic irradiation at low ADR. For the other three species, the value of k in Eq. (9) was between 0.03 and 0.05; for the sheep and the dog (which are reasonably comparable to man in radiation response), the value of R was around 25 R/day. On the basis of the most similar values of LD_{50}, it seems reasonable to assume that for man exposed to ionizing radiation at low ADR, the average daily recovery rate will be approximated by $R_o = \sim 25$ R/day and k = 0.03 to 0.05 days / R [Eq. (9)].

By applying Eqs. (9) and (17) with reasonably conservative choices of constants, it is possible to predict the acute LD_{50} for man exposed to ionizing radiation over a fair range of values of ADR. In choosing the constants to be used in the equations, one caution must be observed. The LD_{50} at low values of ADR is calculated from the net average gain in lethal dose per day, which is the difference between the ADR and the daily recovery calculated from Eq. (9). At low values of ADR, this difference is very small and quite sensitive to small differences in the values of the constants. We strongly recommend that before choices of constants are made, a comprehensive calculation should be made of the effects of variations in the values of the constants on the estimated LD_{50} at various values of ADR.

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Appendix A

METHOD FOR THE DETERMINATION OF THE TOTAL BONE-MARROW CELL COUNT

The principle of this method involves injection of a dose of high-specific-activity ^{59}Fe into the animal, early removal of a specimen of marrow for determining the ratio of ^{59}Fe to bone marrow cells in the specimen, and subsequent determination of the percentage of the injected dose of ^{59}Fe incorporated into circulating erythrocytes. The number of bone marrow cells is then the amount in erythrocytes divided by the ^{59}Fe : cell ratio of the marrow. Although the ^{59}Fe in the marrow cells is taken up primarily by precursors of erythrocytes, the method gives a valid measure of the total number of nucleated cells in the bone marrow, provided that the marrow specimen obtained is actually representative of the entire marrow of the animal.

The ^{59}Fe used was obtained as a stock solution in 0.1 N HCl. The stock solution was diluted with a 9:1 mixture of physiological saline and 0.15 M sodium citrate to give the required concentration and injection volume. For mice, the dose of ^{59}Fe was 1 μCi, given i.p. in 0.2 ml; for sheep, the dose was 40 μCi, given i.v. in 2 ml.

The procedure in mice was as follows: Ten to 20 animals were injected with ^{59}Fe; 6 to 24 hours was allowed for the body distribution of ^{59}Fe to stabilize, and half of the animals then were weighed and anesthesized with ether. A blood sample was taken by heart puncture; the chest cavity was opened to kill the animal while it was still under anesthesia, and the spleen and both femurs were dissected out. The bone marrow was removed from the femurs by cutting off the ends and flushing the interiors with a fixed volume (2 ml) of ice-cold Hank's solution. A part of the marrow suspension obtained was diluted with WBC counting fluid, and a cell count was made in a hemocytometer. One ml of the marrow suspension was measured out into a counting tube for determination of ^{59}Fe content. A measured portion of blood (0.1 to 0.15 ml) was diluted with physiological saline, and the cells were washed by centrifuging and resuspending in saline two or three times. The washed cells and the supernatants from washing were separately analyzed

for ^{59}Fe content, as was the whole spleen. The mice in the other half of the group originally injected with ^{59}Fe were sacrificed and analyzed in a manner similar to the first half, at 72 to 96 hours after injection.

In calculating the number of bone marrow cells, the following assumptions were used: (1) The erythrocyte volume of a mouse is 0.036 ml/g body weight; (2) the plasma volume of the mouse is 0.039 ml/g body weight; and (3) the average volume of blood included in a sample of two femurs equals 0.003 ml. The first assumption is used in calculating the total amount or percentage of the injected dose of ^{59}Fe in the erythrocytes. The third assumption is based on comparison cell counts of marrow suspensions diluted in saline and in WBC counting fluid. It is used in calculating a correction to the amount of ^{59}Fe in the marrow suspension.

Sample calculations for a normal mouse are shown in Table A-1. In making calculations, the values for ^{59}Fe in marrow and erythrocytes from the second sacrifice were averaged, and the difference between the average values at second sacrifice and individual values at first sacrifice was used in computation of total number of bone marrow cells. The mouse used for these calculations had 9.38×10^8 bone marrow cells; adjusted to 22.5 g body weight, the number of cells would be 9.55×10^8 compared with the overall mean of 10.21×10^8 cells for 20 normal mice used in the study. Note that at the second sacrifice, the amount of ^{59}Fe associated with the marrow cells was significant compared with the first sacrifice (11.5%). Without this correction, the estimated number of bone marrow cells would be lower by the same percentage. Studies with mice sacrificed later than 96 hours, up to 10 days after injection of ^{59}Fe, indicate the amount of ^{59}Fe in bone marrow during this time remains relatively constant and is not available for incorporation into erythrocytes.

Table A-1

SAMPLE CALCULATIONS FOR THE ESTIMATION OF TOTAL BONE MARROW CELL COUNT IN MICE

1.	Bone marrow cells/ml marrow suspension	2.56×10^7
2.	^{59}Fe % injected dose/ml marrow suspension	1.058
3.	Correction for ^{59}Fe in blood and extracellular fluid	0.019
4.	Net ^{59}Fe % injected dose/ml marrow suspension	1.039
5.	^{59}Fe % injected dose/10^8 marrow cells	4.059
6.	^{59}Fe % injected dose/10^8 cells (second sacrifice)*	0.468
7.	^{59}Fe % injected dose/10^8 cells, net decrease	3.591
8.	^{59}Fe % injected dose in erythrocytes (first sacrifice)	4.38
9.	^{59}Fe % injected dose in erythrocytes (second sacrifice)*	38.08
10.	^{59}Fe % injected dose in erythrocytes, net gain	33.70
11.	Total number of bone marrow cells (#10/#7)	9.38×10^8
12.	Weight of mouse (g)	22.1
13.	Total number of bone marrow cells/kg	4.24×10^{10}

* Mean of five animals.

The calculations of number of bone marrow cells in sheep are shown in Table A-2. The procedure and calculations are similar in principle to those in the mouse but the following details differ:

(1) Marrow specimens were obtained from the sternum.

(2) The marrow cells were counted with a Coulter counter, rather than in a hemocytometer.

(3) Because of the heavy contamination of marrow specimens with blood, it was impossible to obtain a meaningful analysis of a second marrow specimen obtained after the circulating erythrocytes were labeled with ^{59}Fe.

Since the amount of ^{59}Fe in the circulating erythrocytes reached over 97% of the amount injected, the lack of a second marrow specimen may not be critical for the analysis, but some procedure for obtaining a usable second marrow specimen should be devised for future work. The number of bone marrow cells found for this particular animal was 2.86×10^{10}/kg body weight, and the mean for 25 animals was 2.74×10^{10}/kg. The mean bone marrow cell count for the sheep, adjusted to body weight in kilograms, was approximately 60% of that of the mouse, an encouraging degree of agreement.

The method outlined above gives consistent results but is somewhat difficult to validate by independent means. The agreement between the values obtained for mice and for sheep--two species considerably distant in size and relatedness--are a reasonably strong argument for the validity of the method. Two other considerations, applying primarily to the mouse, are as follows:

(1) If a 22.5-g mouse has 1.69 ml of blood, and the RBC count is $10^7/mm^3$, the total number of erythrocytes is 1.69×10^{10}. If the mean life span of an erythrocyte is approximately 40 days, 4.22×10^8 erythrocytes are produced each day. If 50% of the marrow is erythropoietic, then 5.10×10^8 marrow cells are involved in the production of 4.22×10^8 erythrocytes, and the figure for number of marrow cells appears reasonable.

Table A-2

SAMPLE CALCULATIONS FOR THE ESTIMATION OF TOTAL BONE MARROW CELL COUNT IN SHEEP

1.	Bone marrow cells/ml of suspension	4.50×10^{7}
2.	^{59}Fe % injected dose/ml suspension	3.836×10^{-3}
3.	Correction for blood and extracellular fluid	0.688×10^{-3}
4.	Net ^{59}Fe % injected dose/ml of suspension	3.148×10^{-3}
5.	^{59}Fe % injected dose/10^{10} cells	0.700
6.	^{59}Fe % injected dose in erythrocytes (first sample)	0.24
7.	^{59}Fe % injected dose in erythrocytes (eventual)*	97.26
8.	^{59}Fe % injected dose in erythrocytes, net gain	97.02
9.	Total number of marrow cells (#8/#5)	138.6×10^{10}
10.	Weight of sheep (kg)	48.5
11.	Bone marrow cells/kg	2.86×10^{10}

* Mean of 8 animals.

(2) On the basis of ^{59}Fe labeling, the spleen of the normal mouse contains between 5 and 8% of the total amount of bone marrow in the mouse. When mice are exposed to large single doses (800 R) of X-rays, the spleens become nearly aplastic with respect to bone marrow. Under these conditions, the loss of spleen weight is approximately 48 mg (Krebs, unpublished observations). If the typical marrow cell is spherical, with an average diameter of 10 to 12 μ and a density of 1.0, then a loss of 48 mg in the spleen corresponds to a loss of between 5.3 and 9.2 x 10^7 cells, which is between 5.2 and 9.0% of the estimated total number of bone marrow cells.

These two calculations, combined with the degree of agreement of the estimated number of bone marrow cells between two species, give reasonable confidence that this is a reliable method for estimating the total bone marrow cell count.

After the method had been developed and was being applied to the study of bone marrow cell count after irradiation, it was discovered that Corelli, et al.* had developed another method for the estimation of total bone marrow cell count in mice. Their method is similar to ours in the sense that it uses the ratio of ^{59}Fe to bone marrow cells, but it differs in that the total uptake of ^{59}Fe by the bone marrow cells is estimated by radioactivity determination of the whole, cleaned skeleton. The total number of cells obtained by the method was 6 x 10^8, a value only slightly lower than the one described above (10 x 10^8). The procedure for obtaining a cleaned skeleton involves placing the dried mouse carcasses in colonies of the insect Dermestes frischii, which feeds on the flesh and leaves the bones perfectly clean. The overall procedure is simple in an animal the size of the mouse, but would present difficulties in a larger animal, such as the sheep.

* V. Corelli, G. Briganti, and G. Silini. An analysis of bone marrow erythropoiesis in the mouse. Cell Tissue Kinet. 5, 41-51 (1972).

Appendix B

LD_{50} AND EDR IN MICE

During the years 1965 and 1966, a number of measurements of the 30-day LD_{50} in mice of gamma rays at different values of EDR were made on the radiation range at Camp Parks. The measurements were part of a larger study of the relationships among EDR, rate of recovery, and principal syndrome at death. Part of the study was published previously,* but the remainder was incomplete at the time of disestablishment of the Naval Radiological Defense Laboratory.

The data on the relationship of EDR to LD_{50} are shown in Table B-1. The mice used in the study were CF 1 females obtained from Carworth Farms. They were exposed to the radiation between the ages of 100 and 200 days, and mortality was observed and recorded for 30 days after completion of the radiation exposure. The radiation sources, methods of dosimetry and exposure, and calculation of LD_{50} were the same as those used in the present study.

The values of LD_{50} ranged from 899 R up to 1287 R. At EDR values of 5870 and 3099 R/hr, the values of LD_{50} were not significantly different. At EDR values of 1604 R/hr and less, the values of LD_{50} increased progressively with decreasing values of EDR.

The relationship of EDR to LD_{50} in mice is discussed in Chapter V of this report.

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* J. S. Krebs and G. F. Leong, Effect of exposure rate on the gastrointestinal LD_{50} of mice exposed to ^{60}Co gamma rays or 250 kVp X-rays. Radiat. Res. 42, 601-613 (1970).

Table B-1

MORTALITY AND LD_{50} IN MICE EXPOSED TO ^{60}CO GAMMA RAYS AT VARIOUS VALUES OF EDR

1. EDR = 5,870 R/hr

Dose (R)	Fractional Mortality
701	1/12
729	1/12
775	0/12
782	2/12
840	6/12
885	3/12
934	6/12
944	10/12

LD_{50}: 899 R
C.I.: ± 44 R

2. EDR = 3,091 R/hr

Dose (R)	Fractional Mortality
737	0/36
809	5/36
843	10/36
885	19/36
929	19/36
962	22/36
1,030	36/36
1,111	36/36

LD_{50}: 905 R
C.I.: ± 15 R

3. EDR = 1,604 R/hr

Dose (R)	Fractional Mortality
727	0/30
814	2/30
887	3/30
913	16/30
967	23/30
993	24/30
1,047	27/30
1,158	30/30

LD_{50}: 932 R
C.I.: ± 15 R

4. EDR = 821 R/hr

Dose (R)	Fractional Mortality
784	2/36
864	2/36
937	7/35
1,026	14/36
1,111	30/36

LD_{50}: 1,029 R
C.I.: ± 83 R

5. EDR = 405 R/hr

Dose (R)	Fractional Mortality
816	1/36
917	1/36
1,014	10/36
1,055	14/36
1,156	22/36
1,218	31/36

LD_{50}: 1,095 R
C.I.: ± 27 R

6. EDR = 202 R/hr

Dose (R)	Fractional Mortality
1,210	4/20
1,311	13/20
1,411	18/20
1,512	17/20
1,614	20/20
1,715	21/21

LD_{50}: 1,287 R
C.I.: ± 45 R